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TABLE OF CONTENTS

NEUHAUS, M., Additional data on crossing over between X and Y chromosomes in *Drosophila melanogaster* 333

DOBZHANSKY, TH., Further data on the variation of the Y chromosome in *Drosophila pseudoobscura* 340

SMITH, H. H., Inheritance of corolla color in the cross *Nicotiana Langsdorffii* by *N. Sanderae* 347

SMITH, H. H., The relation between genes affecting size and color in certain species of Nicotiana 361

GORDON, M., Genetics of Platypoecilus III. Inheritance of sex and crossing over of the sex chromosomes in the platyfish . 376

HARNLY, M. H., AND EPHRUSSI, B., Development of eye colors in Drosophila: Time of action of body fluid on cinnabar . . 393

BERG, R. L., The relative roles of stabilization and redifferentiation of the gene in the evolution of the hereditary substance 402

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ADDITIONAL DATA ON CROSSING OVER BETWEEN X AND Y CHROMOSOMES IN DROSOPHILA MELANOGASTER

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Received December 11, 1936

INTRODUCTION

IT HAS been shown (STERN 1929, KAUFMANN 1933, PHILIP 1935, NEUHAUS 1936a, 1936b) that simple crossing over between X and Y occurs both in females and in males of *Drosophila melanogaster*. The present paper is an attempt to ascertain the comparative frequency of such crossing over in both sexes in *D. melanogaster*.

EXPERIMENTAL METHOD

Males with a normal Y and with bobbed in the X were crossed to yellow attached-X Y-short (only the short arm of the Y chromosome). All males from this cross are sterile. However, if a single crossover between X and the short arm of the Y occurs in the father, an X chromosome with the long arm of the Y attached is formed. Sons with such an X are fertile. The appearance of fertile males is thus an indication of the frequency of crossing over in males. In a similar manner, crossing over in males between the X and the long arm of the Y was also studied. For this purpose males were mated to yellow attached-X Y-long (only the long arm of the Y).

The frequency of $\widehat{XX}$ detachments in $\widehat{XX}$Y-short and $\widehat{XX}$Y-long females indicates crossing over between the $\widehat{XX}$ and the short or the long arm of the Y.

RESULTS

The results of these experiments are shown in tables 1 and 2.

TABLE 1

♀♀ $\widehat{XX}$Y-short by ♂♂ XY

Frequency of single crossing over between the X and the short arm of the Y.

IN FEMALES			IN MALES		
Number of flies in F_1	Number of detachments	%±m	Number of males tested for fertility	Number of fertile (XY-long) males	%±m
97829×2=195658	45	$0.0229\times\frac{3}{2}=0.034$ ±0.004	26557	6	0.023±0.009

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TABLE 2

♀♀ $\widehat{XX}$Y-long by ♂♂ XY

Frequency of single crossing over between the X and the long arm of the Y.

IN FEMALES			IN MALES		
Number of flies in F_1	Number of detach-ments	%±m	Number of males tested for fertility	Number of (XY-short) males	%m
125115×2=250230	119	$0.0475\times\frac{3}{2}=0.0711\pm0.0043$	23934	—	—

If crossing over between X and Y is to be compared in both sexes, it is necessary to multiply the percent of crossing over in females by 3/2, since one third of the crossing over, that between both X's, cannot be detected. As is evident from table 1 the frequency of crossing over between X and the short arm of Y is approximately the same in both sexes.

The data in table 2 allow us to conclude that crossing over between X and the long arm of the Y does occur in females but is either never seen or is suppressed in males. This conclusion is also supported by the data to be presented below.

Males having the gene forked and either the short or the long arm of the Y chromosome attached to the proximal end of the X, were mated to attached-X females. If a single crossover between the X and the Y occurs when the X chromosome is divided into two strands, in such a manner that the short arm or the long arm of an X strand exchanges with the "inert" region of the second X strand, attached-X's are formed. A spermatozoon with attached-X's can fertilize a Y-bearing egg so that a forked female with attached-X's is produced. The results are given in tables 3 and 4.

TABLE 3

♀♀ $\widehat{XX}$ by ♂ $\widehat{XY}$-short (forked)

Number of flies in F_1	Number of forked females with attached-X's
79872	66

TABLE 4

♀♀ $\widehat{XX}$ by ♂ $\widehat{XY}$-long (forked)

Number of flies in F_1	Number of forked females with attached-X's
88101	2

These figures also indicate that crossing over in males occurs more frequently between X and the short arm of the Y than between the X and the long arm of the Y. One explanation for this may be that in the presence of the short arm, crossing over between X and the long arm is suppressed. If this is so then, in females $\widehat{XX}$ with a normal Y, crossing over between $\widehat{XX}$ and the long arm is also suppressed and hence the frequency of detachments in those females will be approximately equal to the frequency of detachments in $\widehat{XX}$Y-short females. The data obtained show that there is twice as much breakage in females with a normal Y as in $\widehat{XX}$Y-short females. Consequently, in $\widehat{XX}$Y females crossing over occurs either between the long or the short arms.

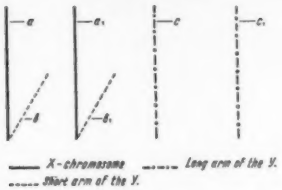
CROSSING OVER BETWEEN PARTS OF THE Y CHROMOSOME

The problem. The two arms of the Y are homologous to the bobbed locus in X. It may therefore be concluded that they are partly allelic, in which case it would be possible to observe crossing over between the long and the short arms of the Y. The above was established by STERN (1929). In a cross of ♀ bobbed by ♂ *bb/Y* STERN found a non-bobbed female. The latter when crossed with males *bb/Y* gave 46 non-bobbed and 74 bobbed females. Some of the non-bobbed females when crossed to *fB* males gave a normal percent of secondary non-disjunction. But the exceptional males were sterile, a fact that attracted STERN's attention. After several crosses he demonstrated that the original female carried an isoarmed Y. The size of these arms is the same as the short arm of the Y. It is possible that this isoarmed Y chromosome arises from crossing over in males between the short arm of one sister strand and the long arm of the second sister strand.

The possibility of crossing over between the short and the long arms or between two short arms in males and in females is analyzed in the following section.

The analysis in males. A method of obtaining attached-X's as a result of crossing over in males between X and the short arm of the Y has been described above. If we compare the frequency of appearance of $\widehat{XX}$ from males $\widehat{XY}$-short Y-long and $\widehat{XY}$-short Y-normal, it is easy to see that the frequency of $\widehat{XX}$ in both groups of males may be different due to crossing over between the short and the long or between two short arms of the Y. This is represented schematically in figures 1 and 2.

In these figures male sex chromatids are shown. Sister chromatids are similarly designated. All possible combinations are given, except sister strand crossing over and crossing over between the X and the long arm, since these very seldom occur. Combinations which give $\widehat{XX}$ are in bold type. The fractions in each column are obtained by dividing the combinations which give $\widehat{XX}$ by the total combinations. Two or three exchanges



For cases when crossing over occurs between the short and the long arms of the Y Chromosome		For cases when crossing over between the short and the long arms of he Y chromosome does not occur	
When crossing over occurs between two short arms	When crossing over between two short arms does not occur	When crossing over occurs between two short arms	When crossing over between two short arms does not occur
1	2	3	4
ab. **ab**$_1$. **ba**$_1$. bc. bc$_1$. a$_1$b$_1$. b$_1$c. b$_1$c$_1$. 2/8	ab. **ab**$_1$. **ba**$_1$. bc. bc$_1$. a$_1$b$_1$. b$_1$c. b$_1$c$_1$. 2/8	ab. **ab**$_1$. **ba**$_1$. a$_1$b$_1$. 2/4	ab. **ab**$_1$. **ba**$_1$. a$_1$b$_1$. 2/4

FIGURE 1.—Method of detecting crossing over between X and Y chromosomes.

which took place in the same cell were excluded from the above calculation because they seldom occur.

If the fractions shown in figure 1 are divided by the corresponding fractions shown in figure 2, the following coefficients are obtained: 2/8:2/20=2.5, 2/8:2/16=2, 2/4:2/12=3 and 2/4:2/8=2. They serve to indicate to what extent the frequency of $\widehat{XX}$ is higher in males $\widehat{XY}$-short Y-long than in males $\widehat{XY}$-short Y-normal.

TABLE 5

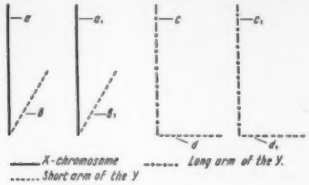
♀♀ $\widehat{XX}$ by ♂♂ $\widehat{XY}$-short Y-long (forked)

Number of flies in F_1	Number of forked females with attached-X's	%±m
35632	40×2=80	0.2245±0.025

TABLE 6

♀♀ $\widehat{yy}$ by ♂ XY-short Y-normal (forked)

Number of flies in F_1	Number of forked females with attached-X's	%±m
78377	24×2=48	0.0613±0.0083



For cases when crossing over occurs between the short and the long arms of the Y chromosome		For cases when crossing over between the short and the long arms of the Y chromosome does not occur	
When crossing over occurs between two short arms	When crossing over between two short arms does not occur	When crossing over occurs between two short arms	When crossing over between two short arms does not occur
1	2	3	4
ab. **ab**$_1$. ad. ad$_1$. **ba**$_1$. bc. bc$_1$. bd. bd$_1$. a$_1$b$_1$. d. a$_1$d$_1$. b$_1$c. b$_1$c$_1$. b$_1$d. b$_1$d$_1$. cd. cd$_1$. dc$_1$. c$_1$d$_1$.	ab. **ab**$_1$. ad. ad$_1$. **ba**$_1$. bc. bc$_1$. bd. bd$_1$. a$_1$b. a$_1$d. a$_1$d. b$_1$c. b$_1$c$_1$b$_1$d. b$_1$d$_1$.	ab. **ab**$_1$. ad. ad,. **ba**$_1$ bd. bd$_1$. a$_1$b$_1$. a$_1$d. a$_1$d$_1$. b$_1$d. b$_1$d$_1$.	ab. **ab**$_1$. ad. ad$_1$. **ba**$_1$. a$_1$b$_1$. a$_1$d. a$_1$d$_1$.
2/20	2/16	2/12	2/8

FIGURE 2.—Method of detecting crossing over between X and Y chromosomes.

In tables 5 and 6 the percent of $\widehat{XX}$ obtained from both types of males is shown. The percent of $\widehat{XX}$ in the first male type is 3.5 times higher than in the second type. Thus it may be concluded that (a) crossing over occurs in males between two short arms and (b) if crossing over between the short and the long arms occurs, its frequency is much lower than crossing over between two short arms or between X and the short arm.

The analysis in females. To measure crossing over between the long and the short arms we compared the percent of detachment of $\widehat{XX}$ in $\widehat{XX}$Y-normal females with the total percent in $\widehat{XX}$Y-short and $\widehat{XX}$Y-long females. The frequencies of detachments were 0.0229 ± 0.0032 and 0.047 ± 0.0043, respectively, making the frequency of detachments in $\widehat{XX}$Y females 0.0747. In practice the percent detachment in $\widehat{XX}$Y females is found to be 0.0506 ± 0.0063, the difference being significant. Such a decrease can be explained as a result of crossing over between the long and the short arms. Indeed, the decrease of detachment in females $\widehat{XX}$Y should be one-third smaller than expectancy.

Likewise, the percent of detachments in females $\widehat{XX}$Y-short Y-short should be one-third less than the percent of detactment in $\widehat{XX}$Y-short fe-

males, if crossing over between two short arms occurs. In practice the frequencies are 0.0227 ± 0.0065 and 0.0229 ± 0.0032 for the first and second respectively. The difference is not significant.

THE DISTRIBUTION OF REGIONS IN X WHICH ARE HOMOLOGOUS TO THE SHORT AND THE LONG ARMS OF Y

Crossing over between X and the short arm. In studying the frequency of crossing over between X and the short arm of Y in males, an X was used which contained bobbed, in order to detect crossing over on the left or right of bobbed. If crossing over takes place to the left of bobbed, then an $\widehat{XY}$-long with the normal allele for bobbed should arise. If crossing over takes place to the right of bobbed, then an $\widehat{XY}$-long containing the original bobbed should be observed.

We obtained six $\widehat{XY}$-long and among these four contained $+^{bb}$ and two contained the original bobbed, indicating that the region in X which is homologous to the short arm lies on both sides of bobbed.

Crossing over between $\widehat{XX}$ and the long arm. The long arm of the Y is known to contain an extreme allele of bobbed. When a detachment occurs in females $\widehat{XX}$Y-long, two free X's are formed. One of them carries an attached long arm and the other remains without any part of the Y attached. The latter should manifest bobbed if crossing over between $\widehat{XX}$ and the long arm occurs to the left of the normal allele for bobbed, and should manifest non-bobbed if crossing over takes place to the right of bobbed.

Eighteen X's without an attached long arm were investigated and all manifested the normal allele for bobbed, indicating that crossing over between X and the long arm takes place only to the right of bobbed. Hence, the region of the X which is homologous to the long arm of the Y lies to the right of bobbed.

SUMMARY AND CONCLUSIONS

1. The frequency of single crossing over between X and the short arm of Y is almost the same in both sexes of *Drosophila melanogaster*. (♀ ♀ 0.034 ± 0.004, ♂ ♂ 0.023 ± 0.009).

2. The frequency of crossing over between X and the long arm of Y in females is 0.071 ± 0.004. In males it is decidedly less.

3. In $\widehat{XX}$Y-normal females crossing over is observed between $\widehat{XX}$ and either the short or the long arms.

4. Crossing over between two short arms takes place in males with the same frequency as between X and the short arm.

5. If crossing over occurs between the short and long arms in males it is much less frequent than crossing over between two short arms.

6. It is very likely that crossing over between the short and the long

arms takes place in females almost as frequently as crossing over between $\widehat{XX}$ and the short arm.

7. Crossing over between X and the short arm occurs either to the left or the right of bobbed. Hence, the regions in X which are homologous to the short arm lie on either side of bobbed.

8. Crossing over between X and the long arm takes place only to the right of bobbed, indicating that the region in X homologous to the long arm is located to the right of bobbed.

NOTE

Since crossover individuals sometimes appear in groups or "bundles," it is necessary to note the following. The six crossovers in table 1 appeared singly in the progenies of six separate males, although the complementary crossovers cannot be detected with the above method. About 30 percent of all detachments of attached-X's arose in twos, threes and other small groups in the progenies of single females. From $\widehat{XY}$-short males, attached-X daughters in groups or "bundles" are sometimes produced.

The question therefore arises whether this casts doubt on some of the conclusions drawn. Assuming that the frequency of crossing over is the same in both sexes, or the same in males $\widehat{XY}$-short Y-long and males $\widehat{XY}$-short Y-normal, then it makes no difference at which stage of oögenesis or spermatogenesis crossing over takes place, the percent of crossing over being the same in all categories of flies. This may be true of course only if the experimental data are significant enough to exclude accidental errors.

The experimental data given in this paper seem to be well founded. Hence even if the phenomenon of "bundles" does affect the conclusions reached in the present study it cannot do so to any great extent.

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FURTHER DATA ON THE VARIATION OF THE Y CHROMOSOME IN DROSOPHILA PSEUDOOBSCURA

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Received January 4, 1937

INTRODUCTION

SIX types of Y chromosome have been recorded in *Drosophila pseudoobscura*, three of them in race A and three others in race B. The six types differ from each other in size and in shape; the characteristics of the Y chromosome present in a given strain are constant. Each type is encountered in populations inhabiting a definite part of the species area.

Since the time of publication of the above observations (DOBZHANSKY 1935) the amount of available data has considerably increased due to accession of material from the hitherto unexplored parts of the area where the species is known to occur. One more (the seventh) type of Y chromosome has been detected, and the geographical distribution of the six previously known ones made clear.

The writer wishes to acknowledge here the help extended to him by many persons during his trips undertaken for the purpose of collecting living strains of Drosophila, and especially by DR. A. C. BAKER of Mexico City, Mexico.

OBSERVATIONS

The technique of collecting wild strains of *D. pseudoobscura* and of studying their chromosomes has been described (DOBZHANSKY 1935). The present series of observations differs from the older one only in that the cytological analysis has been performed in many cases on the first generation of flies hatching in the laboratory from parents collected in nature. This difference may account for several instances of two types of Y chromosome being found in the same strain.

The cytological characteristics of the seven types of Y chromosome are represented semi-diagrammatically in figure 1. The chromosome of type I is the largest in size, and all others may be derived from it by losses of sections. Whether or not they have been actually so derived in the history of the species is a different question. Type VII, which is here first described, resembles type II rather closely. The type VII is however smaller than type II, equal armed, and is known to occur in race A only, while type II has been recorded exclusively in race B. Type VII differs from type VI only in size, but the difference is large enough to make the classification reliable.

A list of strains of *D. pseudoobscura* which are known to carry the Y chromosome of a given type is presented below. This list is to be taken as

an addition to the records published previously (DOBZHANSKY 1935). Each strain in the list is designated by the name of the locality in which its wild ancestor has been captured, and by a serial number. Thus, Charleston-4 refers to the strain No. 4 from Charleston Peak, Nevada. The number of the spermatogonial metaphase plates examined in a given strain is indicated in parentheses.

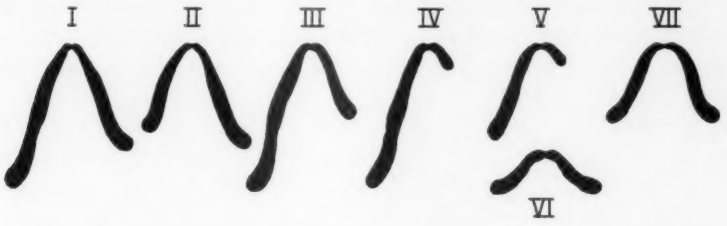

FIGURE 1. The seven types of Y chromosome encountered in *Drosophila pseudoobscura* (semi-diagrammatic).

Type I (Race A)

Arizona: Santa Rita-2 (7), Santa Rita-3 (10), Santa Rita-4 (9), Santa Rita-5 (10), Santa Rita-6 (5).

Mexico: Durango: Otinapa-7 (8); Morelos: Cuernavaca-5 (7); Oaxaca: Cerro San Jose-4 (12), Cerro San Jose-5 (15).

Type I (Race B)

California: Sequoia Park-8 (8), Lake Tahoe-1 (12), Tahoe-5 (15).

Type II (Race B)

Washington: Lake Quinault-13 (6).

Type IV (Race A)

Wyoming: Big Horn-5 (8), Big Horn-6 (8).

California: Tahoe-2 (7), Santa Cruz Island-1 (7), Santa Cruz-2 (7).

Nevada: Charleston-4 (7).

Arizona: Santa Catalina-1 (4), Santa Catalina-2 (7), Santa Catalina-3 (7), Santa Catalina-4 (9), Santa Rita-1 (14), Santa Rita-4 (3), Santa Rita-7 (12), Chiricahua-3 (7), Chiricahua-5 (7).

New Mexico: Pinos Altos-1 (8), Pinos Altos-2 (6), Pinos Altos-3 (8), Pinos Altos-4 (6), Mountains near Carizozo-5 (7), Magdalena-4 (7), Zuni-6 (5), Taos-1 (8).

Colorado: Mesa Verde-1 (5), Mesa Verde-4 (8), San Juan-1 (7).

Mexico, Durango: Otinapa-3 (10); Morelos: Cuernavaca-2 (12), Cuernavaca-5 (13), Cuernavaca-6 (14), Cuernavaca-8 (7); Baja California: Guadalupe (8), Santo Tomas (6).

Type V (Race A)

Idaho: Boise-1 (13), Boise-2 (9).
South Dakota: Black Hills-3 (10), Black Hills-5 (9).
Nebraska: Scottsbluff-3 (6), Scottsbluff-5 (5).
California: Providence Mts.-1 (8).
Nevada: Charleston-1 (6), Las Vegas-1 (3), Las Vegas-2 (5).
Utah: Cedar City-6 (5), Cedar City-9 (8), Cedar City-10 (6), Cedar City-11 (6).
Colorado: Estes Park-1 (10), Estes Park-3 (14), Estes Park-5 (20), University Camp-1 (6), University Camp-2 (8), University Camp-5 (11), Pikes Peak-2 (6), Tree Line on Pikes Peak-1 (10), Tree Line-2 (6), San Juan-4 (9), San Juan-5 (6), San Juan-6 (9), Mesa Verde-3 (6).
Arizona: Gila near Yuma-2 (11), Grand Canyon-1 (5), Grand Canyon-2 (7), Flagstaff-1 (4), Flagstaff-8 (7), Chiricahua-6 (7), Chiricahua-7 (10).
New Mexico: Zuni-2 (10), Zuni-5 (5), Taos-3 (5), Taos-4 (8), Magdalena-1 (6), Magdalena-2 (8), Carizozo-2 (7), Carizozo-4 (7), Carlsbad-1 (7).
Texas: Florence-2 (12).

Type VI (Race A)

Arizona: Chiricahua-2 (4).
New Mexico: Zuni-5 (11), Carizozo-4 (9).
Colorado: San Juan-2 (8), San Juan-3 (12).

Type VII (Race A)

Colorado: Estes Park-2 (16), University Camp-3 (11), University Camp-4 (8).

The available information on the geographical distribution of the seven types of Y chromosome is summarized in map form in figure 2 (for race A) and figure 3 (for race B). It is obvious from these maps that each type is encountered in populations inhabiting a definite area, outside of which it is met with seldom or not at all. In some localities the entire population seems to be homogeneous with respect to the type of Y chromosome, while in other localities mixed populations are found. For instance, in Chiricahua and Zuni mountains (Arizona and New Mexico respectively), and in San Juan mountains (Colorado) as many as three different Y chromosomes are represented.

Type I in race A is restricted to Mexico and to southern Arizona, where it exists side by side with type IV. In Oaxaca, Mexico, type I has been found without type IV, but since only two strains were examined from this region the absence of type IV is by no means certain. Type IV is very widely distributed; it is the only type of Y chromosome found in race A on the Pacific Coast from British Columbia to Baja California, and it

occurs also in southern Arizona, New Mexico, southern part of Colorado, and in Mexico. East of the Sierra Nevada—Cascade mountains and north of Mexico type V is predominant, but two strains from Big Horn mountains,

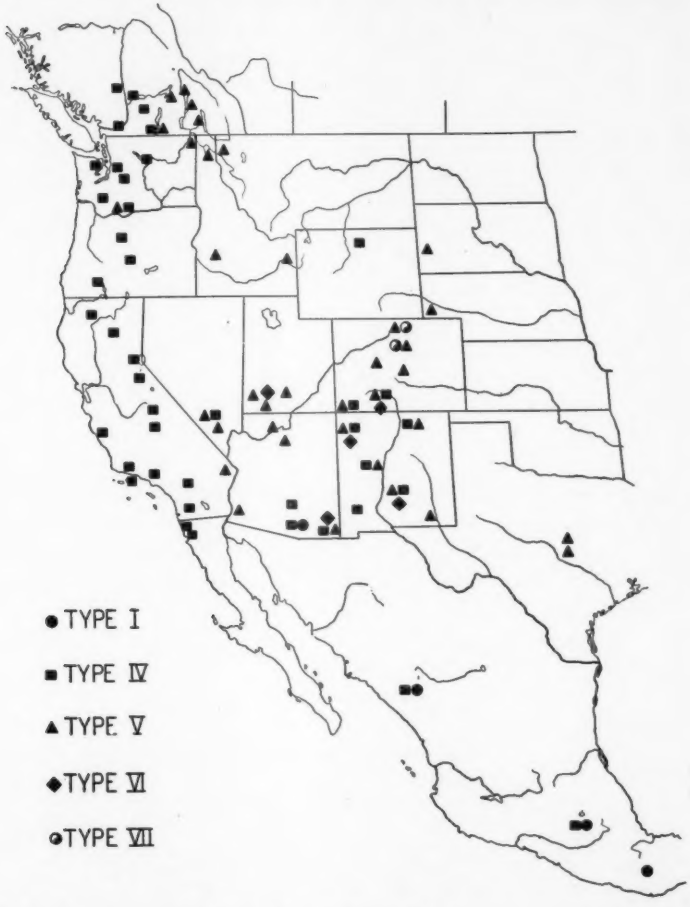

FIGURE 2. The geographical distribution of types of Y chromosome encountered in race A.

well within the territory occupied by type V, were found to possess type IV. Type VI is present as an admixture in the populations from some localities in the Southwest. Type VII is endemic to the highest part of Rocky Mountains, Colorado. In race B the situation is quite simple: type I is present almost throughout the range of the race, type II is restricted to

the region around Puget Sound, and type III to southern Sierra Nevada and southern Coast Ranges.

Race A and race B of *D. pseudoobscura* appear indistinguishable morphologically. Apart from their physiology and their genetic behavior, the Y chromosome was supposed to represent the sole difference between the races. The older data showed (with one perhaps doubtful exception) that types I, II, and III are restricted to race B, and types IV, V, and VI to

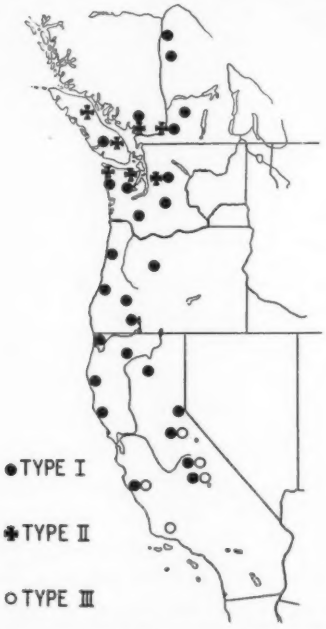

FIGURE 3. The geographical distribution of types of Y chromosome encountered in race B.

race A. At present the Y chromosome can no longer be regarded as a racial differential, since type I (or two types indistinguishable cytologically) occurs in both races. In this connection it is noteworthy that in the rather large area where both races are encountered (compare figures 2 and 3) type I is present in race B but never in race A. In Mexico, where race B does not live, race A may have the Y of type I. It follows that the occurrence of this chromosome in both races cannot be due to interracial hybridization going on in nature at the present time.

DISCUSSION

The geographical variation displayed by the Y chromosome of *Drosophila pseudoobscura* is interesting mainly because the character involved

is in this case a chromosome, and intraspecific variations in the chromosome structure have been only very insufficiently studied from a geographical standpoint. It seems worth while to know that the situation which obtains here is not different from the ubiquitous picture encountered wherever one observes a geographical variation of a character modified by a single or a few genes producing clearly classifiable effects on the phenotype. Populations inhabiting different localities differ in the relative frequencies of the genotypes composing them, although populations in some areas may be homozygous for genes that are totally lacking in other areas. A population or a race can be described adequately only in terms of gene frequencies, not in terms of abstract "racial types."

The fact that Y chromosomes of type I occur in race B as well as in race A is very suggestive. As pointed out above, type I is the largest Y chromosome in the species, and all other types can be derived from it by losses of sections of one or of both limbs, and perhaps by shifts in the location of the spindle fiber attachment. The single close relative of *D. pseudoobscura* living on the American continent, namely *D. miranda*, also has a Y chromosome of type I. One may speculate that type I has been present in the common ancestor of both races of *D. pseudoobscura* and of *D. miranda*. This speculation receives no support if somewhat more distant relatives of our species are taken into account. The European species which FROLOWA and ASTAUROW (1929) take to be *D. obscura* Fall. seems to have a Y chromosome of type IV or V, and the same is true for species of the somewhat more distantly related *affinis* group in America (STURTEVANT and DOBZHANSKY 1936a). Unless the common ancestor of all these species was already variable with respect to Y chromosome, one is forced to assume that seemingly identical types of Y's have arisen independently in different phylogenetic lines. This is a situation which is familiar to many investigators who have attempted to trace phylogenies on the basis of comparative morphological and embryological data.

Comparative chromosome morphology is frequently supposed to furnish especially reliable information for the elucidation of phylogenetic bonds, a supposition founded on an implied but seldom stated assumption that the visible chromosome structure is a more conservative "character" than morphological structures at large. Proofs of the correctness of this assumption are lacking, and some facts argue rather against it. For example, the pair of species *D. melanogaster* and *D. simulans* have very similar gene arrangements in their chromosomes, while the pair *D. pseudoobscura* and *D. miranda* are very different in gene arrangement (PÄTAU 1935, KERKIS 1936, DOBZHANSKY and TAN 1936). And yet the external morphological and the genetic differences (as judged by their behavior in crosses) between the two species of a pair are of the same order of magnitude in

the two cases. The genic differentiation and the differentiation of the chromosome structure do not necessarily go hand in hand. Only very special kinds of chromosome study, those on overlapping inversions (STURTEVANT and DOBZHANSKY 1936b) seem able to produce data of a more exact nature bearing on phylogenetic relationships.

SUMMARY

1. The geographical distribution of the seven types of Y chromosome known in *Drosophila pseudoobscura* is shown in figures 2 and 3.

2. Type I of Y chromosome is encountered in both races of *D. pseudoobscura* as well as in *D. miranda*. The supposition that type I is the ancestral type of Y chromosome leads however to difficulties, since certain other species related to *D. pseudoobscura* have Y's which resemble the types IV and V of *D. pseudoobscura*.

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INHERITANCE OF COROLLA COLOR IN THE CROSS NICOTIANA LANGSDORFFII BY N. SANDERAE[1]

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THE work on color inheritance reported in this paper was done to establish a basis for studies on size inheritance. This problem is part of certain investigations on the inheritance of size being carried on at Harvard University by DOCTOR E. M. EAST. It is still being continued. I am indebted to DOCTOR EAST for guiding me throughout the course of the research work, for his constructive criticisms, and for the greenhouse and field space generously left for my use. I also wish to thank DOCTOR EDGAR ANDERSON for his helpful suggestions.

MATERIAL

Nicotiana Langsdorffii Weinm. and *N. Sanderae* hort., which were used as the parent species in these crosses, differ conspicuously in corolla color and size. *Langsdorffii* is a good taxonomic species, having a green flower about two centimeters long. The plants used were descended from a line which has been inbred for at least fifteen generations by DOCTOR EAST at the BUSSEY INSTITUTION.

The *Sanderae* plants were from a horticultural variety known as Sutton's Scarlet. This strain has an unusually large corolla, about seven centimeters long, which is intensely red in color (somewhat more red than RIDGEWAY'S "amaranth purple"). Although *Sanderae* is of hybrid origin, this variety is very constant in size and color. A communication from Sutton and Sons, dated February 1935, stated that Sutton's Scarlet "is the result of a selection made by us about twenty years ago and grown on annually each year since." Seeds were obtained in 1932 from DOCTOR EDGAR ANDERSON.

The F_1 generation was produced by crossing *Langsdorffii*×*Sanderae*. The reciprocal cross was never successful, apparently because *Langsdorffii* pollen tubes grow too slowly and finally stop altogether near the top of the ovary of Sutton's Scarlet.

N. Langsdorffii is self-fertile, *SfSf*, (EAST 1932). This strain of *Sanderae* had both self-fertile and self-sterile alleles at the *S* locus, but only self-fertile plants were used. Each parent species has nine pairs of chromosomes but some irregularity of chromosome distribution was observed in the F_1

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hybrid. A superficial examination of first and second divisions in the PMC indicated that six chromosomes from each species pair to form normal bivalents. Two lagging univalents are characteristically seen, while the remaining two pairs of chromosomes form some type of configuration, possibly a chain of four. The two chromosome complements may differ by a segmental interchange.

N. Langsdorffii and Sutton's Scarlet each produce from 1 to 25 percent of aborted pollen, but most frequently the percentage is in the lower part of this range. Pollen of the F_1 contains 50 percent sterile grains. The F_2's grown ranged from 1 to 75 percent aborted pollen in different plants. Departures from normal chromosome pairing and distribution are thought to be largely responsible for high percentages of pollen abortion.

The factors influencing corolla color which are considered in these experiments may be grouped into three classes: those affecting (A) anthocyanin color, (B) plastid color, (C) pollen color.

ANTHOCYANIN COLOR

Anthocyanin color is found in the cell sap of epidermal cells of the corolla. There is a basic color gene *C* which, when present in the homozygous recessive condition, inhibits the production of anthocyanin in the stem and flowers. All plants used here were homozygous for the dominant *CC*. The factors listed below express themselves only when *C* is present.

P: a basic gene for the production of anthocyanin color in the inner (upper) surface of the corolla. It probably has, in addition, a minor diluting effect in the heterozygous condition.

I1 and *I2:* independent pairs of genes for increasing color intensity.

D: a dilution (lightening) gene from *Langsdorffii*.

R: a gene for red color; *r* is purple.

E1 and *E2:* independent genes for extension of color onto the outer (lower) surface of the corolla.

The various combinations of these genes give approximately the same phenotypes as the factors postulated by SACHS-SKALINSKA (1921). The interpretation of the genes involved is different, however, with the exception of *I1* and *I2*, which were proposed by her and are in full agreement with my results.

(1) *The P factor.* Plants homozygous for *p* have no color on the inner surface of the corolla. In addition merely to permitting full development of pigment, *P* by itself, or a gene very closely linked to it, produces a purplish-magenta color in the cells of a limited area on the inner surface of the corolla where the tube and limb meet. Constant pH conditions may account for this typical coloration. *N. Sanderae* is *PP*. *N. Langsdorffii* is *pp*. *P* is probably the same as the *I* of BRIEGER (1926, 1935) and the *P* of EAST (1932).

Table 1 shows the segregation of *P–p* in all families in which a 3:1 ratio was expected. The totals show an obvious general tendency for the *pp* class to be deficient in numbers. Although the results fit closer to a 13:3 ratio, there are two reasons why a two gene interpretation is not justified: 1) Colorless segregates when intercrossed do not produce color; 2) The total backcross ratio (table 2) is much nearer a 1:1 than a 3:1. The backcross ratios also show a deficiency in the *pp* class. The best interpretation appears to be that the segregation is monofactorial, but there is some sort of selection in favor of the *PP* and *Pp* groups.

TABLE 1

Families in which the expected segregation is 3P:1pp.

FAMILY NUMBER	n	OBTAINED RESULTS *P*	*pp*	DEV.	P.E.	D./PE
334	139	110	29	−5.75	3.44	1.67
344	238	207	31	−28.50	4.51	6.32
3406	28	23	5	−2.00	1.55	1.29
34015	27	23	4	−2.75	1.52	1.81
34016	28	25	3	−4.00	1.55	2.58
34017	19	14	5	+0.25	1.27	0.19
34018	28	23	5	−2.00	1.55	1.29
3412	95	77	18	−5.75	2.85	2.02
3415	103	86	17	−8.75	2.96	2.96
3421	53	30	23	+9.75	2.13	4.58
3421a	16	14	2	−2.00	1.17	1.71
3422	16	7	9	+5.00	1.17	4.27
3427	26	22	4	−2.50	1.49	1.68
3428a	46	37	9	−2.50	1.98	1.26
3501	50	38	12	−0.50	2.07	0.24
3503	40	30	10	0.00	1.85	0.00
3504	40	33	7	−3.00	1.85	1.62
35011	46	33	13	+1.50	1.98	0.76
35022	28	20	8	+1.00	1.55	0.65
Total	1066	852	214	−52.50	9.60	5.47

(2) *Intensity genes.* Intensity of anthocyanin color is governed chiefly by two independent pairs of dominant genes, *I1* and *I2*, from *Sanderae*. Each pair alone increases the intensity approximately to the same degree, and the two pairs together deepen the color to a still greater extent. *N. Sanderae* is *I1 I1 I2 I2*. *N. Langsdorffii* is *i1 i1 i2 i2*. Additional genes appear to be responsible for masking the intensity segregation in the F_2 and certain fraternities in subsequent generations.

For scoring and recording purposes it was found most convenient to establish four phenotypic classes with respect to intensity: ***very intense***, ***intense***, ***light***, and ***very light***. ***Very intense*** is characteristic of Sutton's Scarlet. ***Intense*** is typified by the F_1. The ***very light*** group has color in the

cells of the inner surface only at the junction of the tube and limb. The *light* class ranges from the intensity of the F_1 to *very light*.

In the F_2 a more or less continuous series is formed from *very intense* to *very light*. Nevertheless, from results in some of the succeeding generations where sharp segregation was found, it seems evident that the arbitrary phenotypic divisions have a significant genetic basis.

Families which segregated clearly into easily classified, discontinuous groups are listed in table 3. By following the table it may be seen that most of the results are capable of explanation on the basis of the two in-

TABLE 2

Families in which the expected segregation is 1Pp:1pp.

FAMILY NUMBER	n	OBTAINED RESULTS *Pp*	*pp*	DEV.	P.E.	D./PE
335	120	67	53	−7.00	3.69	1.90
336	159	79	80	+0.50	4.25	0.11
345	34	20	14	−3.00	1.97	1.52
3407	23	13	10	−1.50	1.62	0.93
3408	24	15	9	−3.00	1.65	1.82
3420	46	27	19	−4.00	2.29	1.75
3420a	89	59	30	−14.50	3.18	4.56
3421	53	30	23	−3.50	2.46	1.42
3422	16	7	9	+1.00	1.35	0.74
3423	94	54	40	−7.00	3.27	2.14
3424	24	13	11	−1.00	1.65	0.61
3428	95	70	25	−22.50	3.29	6.84
3429	13	7	6	−0.50	1.22	0.41
3502	50	27	23	−2.00	2.38	0.84
35012	40	17	23	+3.00	2.13	1.41
Total	880	505	375	−65.00	10.00	6.50

dependent dominant genes, *I1* and *I2*. There is, however, a regrettable paucity of numbers in some cases.

Evidence from families 34018, 3412, 3413 and 3413a indicated that *P* has a lightening effect when heterozygous. 34018 and 3412 both contained colorless (*pp*) and colored (*PP* and *Pp*) segregants, and the proportion of "more intense" to "less intense" among the colored individuals was approximately 1:2. The influence of *P* on intensity is not manifest in the *very intense* and *very light* classes.

Family 349a (*PPI1I1I2i2*×*Langsdorffii*, table 3) was expected to give 1 *very intense:* 1 *intense*. The actual segregation obtained, 1 *light:* 1 *very light*, was interpreted to mean that there is a dominant diluting factor (*D*) in *Langsdorffii*. *P* does not have a great enough effect to cause such a conspicuous shift. Further indications of the dilution gene are: (1) The

F_1 from *Langsdorffii*×Sutton's Scarlet is markedly less intense than Sutton's Scarlet. (2) Backcrosses of the F_1 to Sutton's Scarlet segregate for 1 *very intense* (*dd*): 1 *intense* (*Dd*).

TABLE 3

Families segregating for intensity of anthocyanin color.

FAMILY NUMBER	PARENTS	FACTORS INVOLVED	EXPECTED SEGREGATION	OBTAINED SEG.	DEV.	P.E.	D./PE
3402	334–3 selfed	*PPIiiIiIiIi2i2*	9 *very intense*	16	0.25	1.77	0.71
			6 *intense*	11	0.50	1.73	0.29
			1 *light*	1	0.75	0.86	0.87
348	3402–3 selfed	*PPIiiIiIi2i2*	3 *intense*	18	1.50	1.49	1.00
			1 *light*	8			
349	3402–11 selfed	*PPIiIiIiIi2i2*	3 *very intense*	26	2.25	1.63	1.38
			1 *intense*	5			
3410 &	3402–3 recipro-	*PPIiiIiIi2i2*	1 *very intense*	77	0		0
3410a	cally×3402–11	*PPIiIiIiIi2i2*	1 *intense*	77			
35021	3410a–72	*PPIiIiIiIi2i2*	1 *very intense*	8	0		0
	×3425–42	*PPiiiIiIi2i2*	1 *intense*	8			
349a	3402–11	*PPIiIiIiIi2i2dd*	1 *light*	22	3.50	2.41	1.45
	×*N. Langs.*	*ppiiiIiIi2i2DD*	1 *very light*	29			
34018	334–24 selfed	*PpIiIiIiIi2i2*	1 *intense*+	10	2.60	1.49	1.75
			2 *intense*−	12			
3412	34018–18 selfed	*PpIiIiIiIi2i2*	1 *intense*+	28	2.30	2.79	0.82
			2 *intense*−	49			
3413 &	34018–18 recipro-	*PpIiIiIiIi2i2*	1 *intense*	107	3.00	4.86	0.62
3413a	cally×3402–28	*PPIiIiIiIi2i2*	1 *light*	101			
3503	3412a–14 selfed	*PpIiIiIiIi2i2*	3 *very intense*	20	2.50	1.60	1.56
			1 *intense*	10			
3504	3412a–45 selfed	*PpIiIiIiIi2i2*	3 *very intense*	22	2.75	1.68	1.64
			1 *intense*	11			
3415	34017–7 selfed	*Ppiiiiiii2i2*	3 *very light*	87			
			1 *colorless*	17			
35011	3419–10* selfed	*PpIiiiIiIi2i2*	1 *very light*	3	0.94	0.94	1.00
			15 all other intensities	30			
35012	3419–10	*PpIiiiIiIi2i2*	1 *very light*	4	0.25	1.20	0.21
	×3415–42	*ppiiiiiii2i2*	3 all other intensities	13			
3536	35021–16 selfed	*PPIiiiIiIi2i2*	1 *very light*	5	0.06	1.47	0.04
			15 all other intensities	76			
3538	35021–16	*PPIiiiIiIi2i2*	1 *very intense*	11	1.75	1.78	0.98
	×35015–3†	×*PPiiiiiii2i2*	2 *intense*	14	4.50	2.05	2.19
			1 *very light*	12	2.75	1.78	1.55

* 3419=34017–7×Sutton's Scarlet

† 35015=3425–42 selfed.

On the basis of the above analysis, expected ratios in the F_2 were compared with the results obtained. They are tabulated below for family 344.

Expected ratio	9 *very intense:*	33 *intense:*	18 *light:*	4 *very light*
Obtained numbers.	29	112	44	22
Expected numbers.	29	107	58	13

The agreement between expected and obtained results is only fairly good. However, the lightening effect of *Pp* was not considered in computing the expected ratios, and it is the lightest class which is conspicuously large. Still other genes may affect intensity of anthocyanin color in this material.

(3) *Red flower color.* BRIEGER (1935) found that "The red color of *N. Forgetiana* is mainly due to one dominant gene *R*. The recessive gene *r* is present in all other *Sanderae* types and in *Langsdorffii;* and causes a magenta flower color. Both genes are effective only in the presence of the two basic colors *C* and *I*(*P*)."

My results agree, in the main, with those of BRIEGER. It should be pointed out that there are three important considerations to be taken into account in obtaining and analyzing data on color.

1) Almost all the flowers, both red and purple (magenta), had in my material a purplish-magenta region at the junction of the tube and limb on the upper (inner) side of the corolla. In a flower with the minimum of color intensity, this region alone remains colored. Since it is always purple, these *very light* flowers, if considered together with the other data, would give an incorrectly large proportion of purple-flowered plants. Therefore, only the three most intense groups were used in establishing the relative proportions of red and purple offspring.

2) In families which segregated for red and purple, some plants were almost always found to be intermediate in color. These were scored as "purplish-red." Since the most clear-cut, and it appears the most significant, segregation was between reddish flowers and those with no red (purple), both red and purplish-red were grouped together.

3) Changes in environment affect anthocyanin color. Full expression of red is attained in the flowers of plants grown out-of-doors during the summer. In the greenhouse during the winter the same plants may have flowers with a more purplish hue.

Two F_2 families grown in the field during the summer yielded 277 plants in the three most intensely colored classes. Of these, 201 were red; 76 were purple. The deviation is 1.3 times the probable error of a 3:1 ratio. Thirty of the red-flowered plants were scored as "purplish-red."

Modifying factors are no doubt effective in masking clear segregation of the basic *R* gene.

Table 4 shows the results obtained in seven families from selfings and two backcrosses. All except 3427 were grown in the green house during the

TABLE 4

Families segregating for red (R) and purple (r).

FAMILY NUMBER	n	EXPECTED RATIO	OBTAINED RESULTS RED	PURPLE	DEV.	P.E.	D./PE
3427	22	3*R*:1*r*	18	4	1.25	1.37	0.91
3501	38	3*R*:1*r*	29	9	0.50	1.80	0.28
3503	30	3*R*:1*r*	23	7	0.50	1.60	0.31
3504	33	3*R*:1*r*	23	10	1.75	1.68	1.04
3509	41	3*R*:1*r*	32	9	1.25	2.04	0.61
35011	30	3*R*:1*r*	22	8	0.50	1.60	0.31
35013	44	3*R*:1*r*	34	10	1.00	1.94	0.52
3502	27	1*R*:1*r*	17	10	3.50	1.75	2.00
35012	13	1*R*:1*r*	7	6	0.50	1.22	0.41

same winter. The recorded numbers in each instance agree closely with the expected ratios.

(4) *Extension genes.* The two dominant independent genes, *E1* and *E2*, which govern extension of anthocyanin pigment are contributed by *N. Sanderae*. When either one or both are present, there is color on the outside of the corolla. When both are absent, there is no anthocyanin color in the tube and, if any remains on the back of the limb, it takes on a peculiar mottled appearance.

Two hundred and seven colored F_2 offspring obtained from selfing the F_1 (*E1e1E2e2*) between *Langsdorffii* and *Sanderae* gave 187 with color on the outside of the corolla to 20 with no extended pigmentation. The deviation of this proportion from a 15:1 ratio is 2.97 times the probable error.

As predicted, some selfings in subsequent generations yielded 15:1 ratios

TABLE 5

Families segregating for extension factors, E1 and E2.

FAMILY NUMBER	GENES INVOLVED	n	*E*	*e*	DEV.	P.E.	D./PE
3402	*E1e1e2e2* selfed	28	15	13	6.00	1.55	3.87
3505	*E1e1e2e2* selfed	42	32	10	0.50	1.89	0.27
35011	*E1e1e2e2* selfed	33	20	13	4.75	1.68	2.83
3425	*E1e1e2e2* selfed	60	43	17	2.26	2.00	1.13
3427	*E1e1E2e2* selfed	22	21	1	0.38	0.77	0.49
3503	*E1e1E2e2* selfed	30	29	1	0.88	0.89	1.00
3501	*E1e1E2e2* selfed	38	35	3	0.62	1.01	0.61
3403 & 3404	*E1e1e2e2*×*e1e1e2e2* Reciprocally	48	28	20	4.00	2.34	1.71
3507	*E1e1e2e2*×*e1e1e2e2*	50	28	22	3.00	2.38	1.26
3508	*E1e1e2e2*×*e1e1e2e2*	50	29	21	4.00	2.38	1.68
35012	*E1e1e2e2*×*e1e1e2e2*	17	9	8	0.50	1.39	0.30
35013	*E1e1e2e2*×*e1e1e2e2*	50	26	24	1.00	2.38	0.42

while others gave 3:1 ratios (table 5). In two instances, parents which when selfed resulted in 3:1 ratios (3505 and 35011) gave 1:1 ratios 3507 and 35013, respectively) in backcrosses to a homozygous recessive.

Family 3402 deviated significantly from a 3:1 ratio, but the reciprocal backcrosses (3403 and 3404) of the parent to *Langsdorffii* gave a 1:1 ratio.

PLASTID COLOR

(1) *The basic chloroplast gene, G.* Chlorophyll-containing plastids are abundant in the epidermal and deeper layers of cells in the corolla of *N.*

TABLE 6

Families segregating for the basic chloroplast factor, G.

FAMILY NUMBER	n	EXPECTED RATIO	OBTAINED RESULTS		DEV.	P.E.	D./PE
			G	*g*			
334	139	3:1	94	45	−10.25	3.44	2.98
34010	28	3:1	27	1	−6.00	1.55	3.87
34014	28	3:1	23	5	−2.00	1.55	1.29
34017	19	3:1	15	4	−0.75	1.27	0.59
34014–13 selfed	22	3:1	19	3	−2.50	1.37	1.82
34017–19 selfed	25	3:1	21	4	−2.25	1.46	1.54
344	238	3:1	198	40	−19.40	4.51	4.32
3426	53	3:1	31	21	+7.75	2.13	3.64
3501	50	3:1	32	8	−4.50	2.07	2.17
3505	42	3:1	39	3	−7.25	1.89	3.84
35022	28	3:1	26	2	−5.00	1.55	3.23
3502	50	1:1	31	19	−6.00	2.38	2.52
3507	50	1:1	31	19	−6.00	2.38	2.52
3508	50	1:1	24	26	+1.00	2.38	0.42
35010	50	1:1	34	16	−9.00	2.38	3.78

Langsdorffii. N. Sanderae flowers have little chlorophyll, and this small amount is concentrated toward the lower part of the tube and along large veins on the back of the limb.

Previous investigators have presented data which indicated that plastid color is due largely to a single dominant, *G*, from *Langsdorffii*. My data, in the main, substantiate this interpretation. Plants with *gg* have non-green corollas, while those with *G* have flowers of various shades of green. The effect of the basic chloroplast gene is most pronounced in the upper surface of the corolla.

EAST (1916) and BRIEGER (1935) pointed out that an excess of greenish offspring was found in populations obtained from selfing heterozygotes. My data showed similar results (table 6). The large deviations from expected numbers may be due, at least in part, to irregular chromosomal conditions. Evidence for this conclusion will be presented later. Four back-

cross families did not give an excess of non-green plants as EAST's and BRIEGER's results did.

(2) *Modifying factors.* Intermediate degrees of greenness are caused by modifying factors which as yet have not been analyzed satisfactorily. Green segregates in populations obtained from plants that were heterozygous or homozygous for *G* may be all of one shade of green or of varying shades of green, depending on whether the parents were homozygous or heterozygous for modifying factors. Since *G* is, for all practical purposes, a complete dominant, and the original F_1 is intermediate in plastid pigmentation, it follows that the modifying factors must either lack dominance or interact with *G* to inhibit the amount of chlorophyll produced.

Green segregants have been arbitrarily placed into three groups: green, yellow, and cream. Some evidence has been obtained which indicated that two complementary modifying factors, *Y1* and *Y2*, interact with *G* to cause these intermediate shades.

POLLEN COLOR

Blue Pollen, B1B2. N. Sanderae pollen is colored ivory-white. *Langsdorffii* has blue pollen, which is a sporophytic characteristic due to a bluish tapetal deposit.

EAST (1932) first interpreted the inheritance of blue pollen as: "due to the interaction of three independent complementary factors *CBD*. The factor *D* (with *C*) apparently does not produce blue pollen." ANDERSON and DE WINTON (1931) had previously concluded that "probably not more than two or three pairs of factors are involved." BRIEGER (1935) explained his data on the basis of "two complementary genes, *B1* and *B2*."

Since all my plants were homozygous for *C*, there are no data with which to test the validity of the three gene interpretation. All my results may be explained on the basis of two genes, both of which are necessary for the production of blue pollen. The data of EAST (1932) on backcrosses of heterozygous females to homozygous recessive pollen parents were very close to the 1 blue: 3 white ratio expected on a bifactorial scheme, as he has shown.

The symbols *B1* and *B2* will be used here in keeping with the method of nomenclature in multiple factor groups discussed previously in this paper. *N. Sanderae* is *b1b1b2b2*, and breeds true for ivory-white pollen. *Langsdorffii* is *B1B1B2B2*, and breeds true for dark blue pollen color. The F_1 has light blue pollen. This intermediate shade may be due partly to the heterozygous condition of *B1* and *B2*, although there are definite indications of additional modifying factors (or a factor) which affect blueness of pollen. Some populations from selfings consisted of all blue pollen segregants, showing that the parent plant must have been *B1B1B2B2*; yet the

pollen varied in color from dark blue to very light blue in different plants.

In table 7 are listed all the families which could be classified easily into blue and white pollen types. The agreement between expected and obtained results is close in practically every case.

In table 7, 338 and 346 are backcrosses of the original F_1, used as the female, to *N. Sanderae*. The ratio is approximately 1 blue: 3 white. The following data in two families of the reciprocal cross, using the F_1 as the pollen parent, show a very different ratio, namely: 3 blue: 149 white.

Inasmuch as it has proved impossible to get *Langsdorffii* pollen to grow down and fertilize the ovules in the original cross, it seems not unlikely

TABLE 7

Families segregating for blue pollen color factors, B1 and B2.

FAMILY NUMBER	EXPECTED RATIO	GENES INVOLVED	n	BLUE	WHITE	DEV.	P.E.	D./PE
344	9:7	*B1b1B2b2* selfed	84	49	35	1.75	3.07	0.57
344a	9:7	*B1b1B2b2* selfed	79	47	32	3.00	2.97	1.00
344b	9:7	*B1b1B2b2* selfed	41	24	17	0.92	2.14	0.43
3402	9:7	*B1b1B2b2* selfed	26	14	12	0.66	1.71	0.48
3406	9:7	*B1b1B2b2* selfed	26	15	11	0.34	1.71	0.20
3425	9:7	*B1b1B2b2* selfed	44	28	16	3.25	2.22	1.46
3427	9:7	*B1b1B2b2* selfed	15	10	5	1.51	1.30	1.12
3503	9:7	*B1b1B2b2* selfed	25	9	16	5.10	1.67	3.05
3504	9:7	*B1b1B2b2* selfed	34	15	19	4.10	1.95	2.10
338	1:3	*B1b1B2b2*×*b1b1b2b2*	74	28	46	9.50	2.51	3.78
346	1:3	*B1b1B2b2*×*b1b1b2b2*	23	4	19	1.75	1.40	1.25
3405	1:3	*B1b1B2b2*×*b1b1b2b2*	18	3	15	1.50	1.24	1.22
3409	1:3	*B1b1B2b2*×*b1b1b2b2*	25	8	17	1.75	1.46	1.20
3505	3:1	*B1B1B2b2* selfed	42	28	14	4.50	1.89	2.38
3502a	3:1	*B1B1B2b2* selfed	28	22	6	1.00	1.55	0.65
3506	1:1	*B1B1b2b2*×*B1B1B2b2*	46	19	27	4.00	2.29	1.75

that pollen tubes carrying the *Sf* allele from *Langsdorffii* are still unable to grow down the style of Sutton's Scarlet, in time to fertilize the ovules, when they are introduced with F_1 pollen. ANDERSON and DE WINTON (1931), EAST (1932), and BRIEGER (1935) have all pointed out the linkage between a pollen color factor and the sterility factors. Brieger estimated that *S* and *b1* are about 20 crossover units apart in the first linkage group. Therefore, in the cross *N. Sanderae* (*b1b1b2b2*)×F_1(*B1Sfb1B2b2*), the only successful pollen tubes carrying *B1* would be the 10 percent which, as a result of crossing over, are no longer on the same chromosome as *Sf*. Blue pollen would be formed only when *B1* and *B2* grow down in the same nucleus.

Chance of *B1* fertilizing ovules is 1/10.

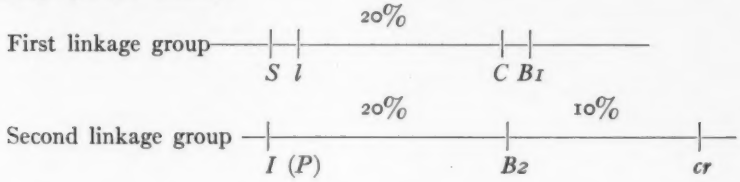
Chance of *B2* fertilizing ovules is 1/2.

Chance of *B1* and *B2* together fertilizing ovules is 1/20.

Therefore, one would expect from this cross 1 blue: 20 white. The deviation of the results is 1.83 times the probable error of this ratio.

LINKAGES BETWEEN QUALITATIVE FACTORS

BRIEGER (1935) has summarized the data on linkages in *Langsdorffii* and *Sanderae*, and established with some degree of certainty the first two linkage groups, namely:



S is the locus of the self-sterility allele.

l is a lethal.

C is the basic gene for anthocyanin color.

cr (crassa) is a recessive gene causing a peculiar type of growth.

The genes for green plastid color and for red anthocyanin pigment were found to be independent of each other and of all the other genes investigated at that time.

My data are neither sufficient in numbers nor are they from types of crosses favorable for establishing, with any degree of accuracy, the crossover values between linked genes. Certain results are presented below because they indicate possible linkages.

The only linkage established by BRIEGER to which additional figures are contributed concerns the relationship between *B2* and *P(I)*. Five families with expected ratios of 3 *P*:1 *p* and 9 blue pollen:7 white pollen, gave the following results: 148 *P*-blue:120 *P*-white:22 *p*-blue:10 *p*-white. From the tables of IMMER (1930), the crossover value was found to be 33.5% ±6.4%. The magnitude of the probable error shows that not much weight can be given to these results. Nevertheless, they do indicate that there is a linkage as described by BRIEGER.

Pollen color and intensity of anthocyanin color were found to be associated in such a way that the proportion of plants with blue pollen increases in progressively less intense classes. This is shown in table 8. It may be interpreted to mean that at least one of the genes affecting intensity (*I1*, *I2*, or *d*) is located in the first or second linkage group.

Possible evidence of linkages between extension genes and intensity was found in the five families listed in table 9. Plants with the *E* genes are, in general, more numerous in the more intense groups.

TABLE 8

Association between pollen color and intensity.

FAMILY NUMBER	VERY INTENSE		INTENSE		LIGHT		VERY LIGHT		n
	BLUE	WHITE	BLUE	WHITE	BLUE	WHITE	BLUE	WHITE	
338	8	20	14	21	6	5			74
3402	7	7	6	5					25
344	7	22	60	52	31	13	12	7	204
345	0	7	3	12	1	0			23
3503	4	9	3	3					19
3504	3	10	7	7					27
Total	29	75	93	100	38	18	12	7	372

Since both pollen and extension genes were found to have some relation to intensity, it is predictable that they should show an association between each other. From the small amount of data available it can be stated that there was such an association.

Evidence has been presented that probably at least one intensity gene and at least one extension gene are located in the same chromosome with a gene for pollen color. This chromosome includes one of the first two linkage groups, probably the first, since no association was found between *P* and either of the gene groups.

Table 10 shows the pronounced association between *P* and a gene which lessens plastid color. Since the factors modifying green have not yet been analyzed satisfactorily the theoretical ratios were not known and all families, both selfs and backcrosses, have been grouped in the totals. In any one family the segregation was between green and yellow or between yellow and cream, depending on the background of other genes. All segregation was clearcut and easily classified.

With the possible exception of *R* and one of the *E* genes, each of the

TABLE 9

Association between extension factors and intensity.

FAMILY NUMBER	VERY INTENSE		INTENSE		LIGHT		n
	E	*e*	*E*	*e*	*E*	*e*	
3402	14	3	2	9	0	1	29
344	29	0	105	7	34	10	185
35011	5	1	9	0	5	8	28
35012	0	0	3	0	5	4	12
35013	2	0	3	0	19	17	41
Total	50	4	122	16	63	40	295

other genes studied in these experiments appeared to be independent of all the rest.

IDENTIFICATION OF COLOR GENES WITH CHROMOSOMES

Since F_1's between *Langsdorffii* and Sutton's Scarlet show abnormalities in chromosome behavior and also produce 50 percent ± aborted pollen, these two phenomena have been considered to bear a causal relation to each other.

TABLE 10

Association between P and a factor modifying plastid color.

FAMILY NUMBER	*P*-GREENISH	*P*-LESS GREENISH	*p*-GREENISH	*p*-LESS GREENISH	n
34015	15	8	1	3	27
3422	0	7	7	2	16
3423	5	49	34	6	94
3428	2	68	18	7	95
3428a	17	20	7	2	46
3519	0	13	7	3	23
3520	3	39	17	1	60
3523	1	13	13	0	27
Total	43	217	104	24	388

Five different segregants were crossed back to Sutton's Scarlet (*gg*). Three of these F_1's had proportionally few aborted grains, from 5 to 20 percent. The other two had from 30 to 50 percent. The parents of the former had in common the plastid factor *gg;* the two latter parent segregants were both *GG*.

Family 35010, produced by crossing $Gg \times gg$, gave the following segregation for plastid color and percent of aborted pollen grains:

	Gg	*gg*
30–50 percent	23	1
5–25 percent	11	15

There is an obvious association between *G* and a high percentage of abortion. If an error of about 15 percent is allowed for estimating the amount of aborted pollen and the overlapping classes are not considered, the association becomes more pronounced.

	Gg	*gg*
35–50 percent	16	0
5–20 percent	6	15

It is reasonable to interpret these results as an indication that *G* is located in one of the interchanged chromosomes, or possibly in the lagging univalent. Populations which segregated for *P*, as well as those segregating

for *G*, often gave ratios that deviated significantly from those expected on chance distribution and recombination. It is likely that this gene also may be located in or affected by one of the chromosomes which does not pair normally.

SUMMARY

Genetic data, from the cross *Nicotiana Langsdorffii*×*N. Sanderae* (Sutton's Scarlet), was presented to show the manner of inheritance of the following color genes:

P, a basic gene from *Sanderae*, governs the presence of anthocyanin color in the upper surface of the corolla.

I1 and *I2*, two dominant, independent cumulative genes from *Sanderae*, are responsible for most of the segregation of anthocyanin pigment intensity. *P* has a minor lightening effect when heterozygous. A fourth gene, *D*, from *Langsdorffii*, has a diluting effect.

R, from *Sanderae*, produces red flower color; *rr* is purple.

E1 and *E2*, from *Sanderae*, govern the extension of color onto the outside of the corolla. Only the double homozygous recessive shows restricted pigmentation.

G, from *Langsdorffii*, controls the presence of chloroplast color. Other factors (*Y1* and *Y2*) may modify the shade of green or yellow.

B1 and *B2*, two complementary genes from *Langsdorffii*, cause blue pollen.

Additional evidence was given supporting the linkage between *B1* and the self-sterility locus.

Genic associations, indicating possible linkages, were demonstrated between pollen color and intensity, extension and intensity, and between *P* and a plastid modifier.

G may be located on a chromosome which does not pair in the normal manner.

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THE RELATION BETWEEN GENES AFFECTING SIZE AND COLOR IN CERTAIN SPECIES OF NICOTIANA[1]

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INTRODUCTION

HEREDITARY factors which govern size are of interest in genetic theory because they usually exhibit a complex type of inheritance. They are of economic importance since such problems as yield of grains, weight of livestock, and size of fruits are involved. Unfortunately, many contributing causes make size characters more difficult to analyze than qualitative characters like color. For example:

1. Size is often affected by environment.
2. Separation into discontinuous classes is usually not obtained.
3. Many size genes appear to lack dominance.
4. Little, if anything, is known about the interaction of these genes.
5. There are probably a large number of genes affecting the size of most organs and organisms.
6. The various genes concerned all affect the same characteristic, although they may act differently during development.
7. It is necessary to select a measurement of size in order to obtain data. This selected standard may not reveal the true genetic situation. The treatment of the data is also important.

In spite of these difficulties, some significant contributions have been made to our knowledge of the inheritance of quantitative characters. From 1909, when NILSSON-EHLE and EAST independently promulgated the multiple factor hypothesis, until about 1923, the analysis of size inheritance was carried out almost entirely by a purely statistical treatment of data. The most valuable additions to our knowledge of quantitative inheritance, from the economic as well as from the theoretical point of view, were concerned with the development of the conception of multiple cumulative factors governing size.

There was no advance in thought or experimental technique in published work on size inheritance until investigations on linkages with qualitative characters began. SAX (1923), the first one to set up an experiment to test genetic associations between color factors and size, found that in a segregating F_2 population, beans having a pigment factor, P, were heavier

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on the average than the white segregants. Carrying out this same type of analysis, linkages between qualitative and quantitative characters were reported in tomatoes (LINDSTROM 1926), maize (LINDSTROM 1931), peas (RASMUSSON 1927), barley (WEXELSON 1933, 1934), Drosophila (WARREN 1924), and mice (GREEN 1931, 1933).

All researches on multiple quantitative factors have shown them to be cumulative in effect. As to the question of how these gene-values accumulate, little is known. Two possible means have been suggested: (1) they are simply additive, though not necessarily by uninvolved arithmetical increments, each gene contributing an individual unchanging amount; and (2) they interact so that the contribution of each gene is dependent on the number and nature of the other size genes present. The results of WEXELSON (1933) support the former interpretation, and those of RICHEY and SPRAGUE (1931) indicate that at least some of the factors have an additive effect.

On the other hand, RASMUSSON (1933) has formulated an interaction hypothesis, based largely on the experience of Svalöf plant geneticists, in which he assumes that "the effect of each factor on the genotype is dependent on all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction." He reported (1935) confirmation of this hypothesis from the interaction of factors for internode length and flowering time in peas.

It seems quite probable that some factors have an additive effect and that others interact.

TREATMENT AND GENERAL STATISTICS

Formulation of a size index

Nicotiana Langsdorffii, which was used as one of the original parents in these studies has a much smaller corolla than that of *N. Sanderae* (Sutton's Scarlet), the other parent species. Two measurements, designated as tube length and length of the maximum lobe, as shown in figure 1, were made on each corolla. EAST (1913; 1916a, b) has shown that tube length is a valuable measurement for size studies in Nicotiana since it is practically unaffected by external conditions; and, most certainly, a single significant measure is preferable to any index. However, the differences dealt with in linkages between genes affecting corolla size and color were often so small that it was thought advisable to combine lobe length and tube length into an index in order to formulate a more inclusive picture of the size differences involved. The coefficient of correlation between lobe length and tube length, as calculated from F_2 data, is $+0.58 \pm 0.03$. Since the tube is much longer than the lobe, it is probably best to obtain the geometric mean between the two in order to arrive at an index of size.

Another problem in the treatment of quantitative data is the determination of a scale by which to compare the values obtained. The following considerations led to the conclusion that a logarithmic scale was preferable to an arithmetic one.

1. The frequency distribution of tube length measurements in *N. Langsdorffii*, plotted in millimeters, shows a steep, narrow curve not at all comparable to that of the larger parent, *N. Sanderae* (figure 2). Since both

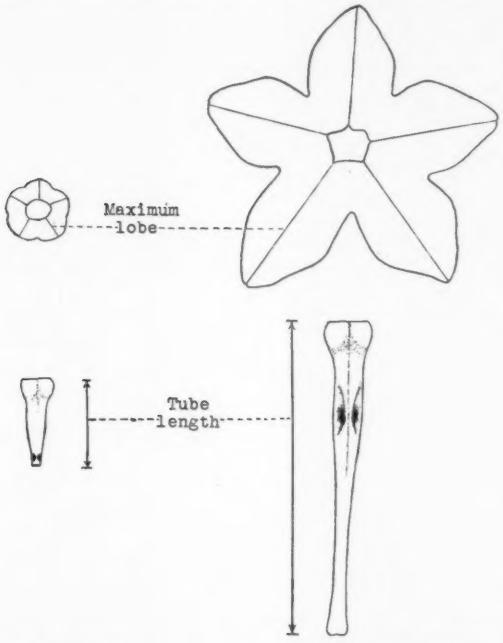

FIGURE 1. Two measurements of corolla size used in these experiments.

parents have been inbred for a number of generations they should be of comparable homozygosity and form approximately the same curve of variability. Comparison of the frequency distributions of the two species on a logarithmic scale, base 10, makes them approximately equally variable (figure 3). *N. Sanderae* still has a somewhat greater variability, which is probably a true picture of the genetic situation due to the presence of self-sterility factors in this species.

2. Growth involves essentially a multiplication of living substance. On the logarithmic scale, equal divisions on the graph signify equal amounts of multiplication; while, on the contrary, on the arithmetic scale they in-

dicate equal additions. Size is the "end product" of growth. Therefore, the treatment of size data on a logarithmic scale gives a more representative picture of the biological phenomenon concerned.

Two considerations in formulating a suitable size index of the corolla

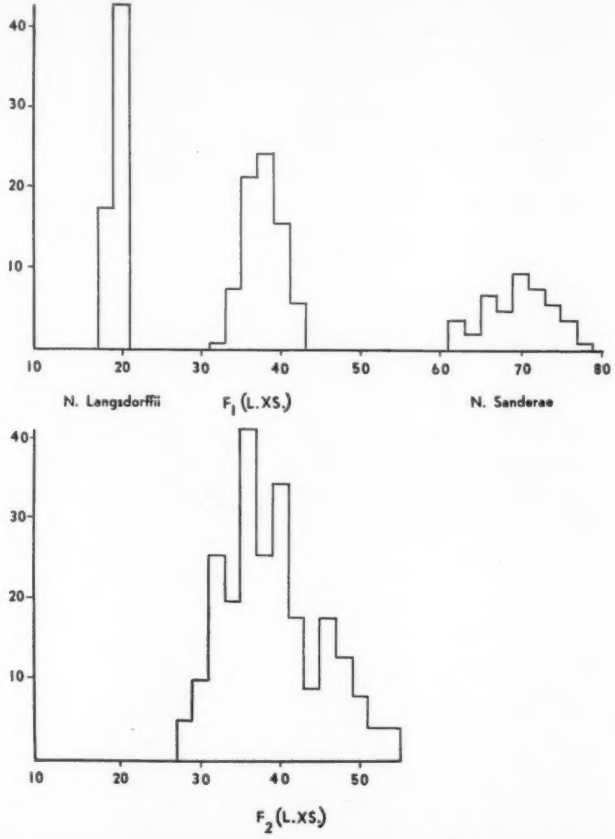

FIGURE 2. Frequency histograms of measurements in millimeters of corolla tube length in *N. Langsdorffii*, *N. Sanderae*, the F_1, and the F_2 generation.

have been discussed, namely: (1) combining the two measurements, tube length and length of the maximum lobe, in a geometric mean; and (2) a logarithmic treatment of this mean. The size index, therefore, is log tube length+log maximum lobe length/2; or, more conveniently, 1000×(log tube length+log maximum lobe length). The division by 2 is eliminated because the paramount interest in these experiments is in comparative

rather than actual values. The product is multiplied by 1000 to do away with an unnecessary use of decimals. Size will be expressed in units of this index.

General statistics

A comparison of standard deviations (table 1) shows that *N. Sanderae* is more variable than *Langsdorffii*, and the F_1 is intermediate. The increase

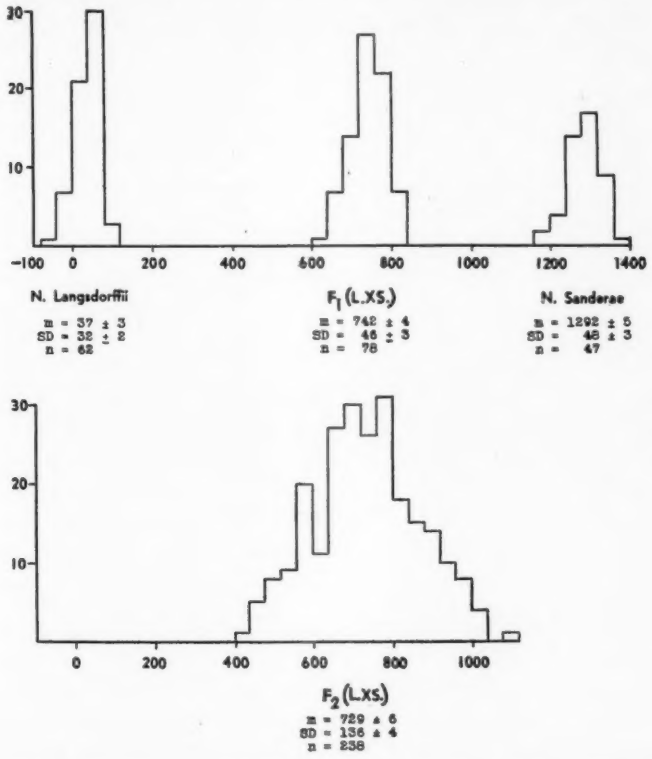

FIGURE 3. Frequency histograms of corolla size index, 1000×(log. tube length+log. maximum lobe length), in *N. Langsdorffii*, *N. Sanderae*, the F_1, and the F_2 generation.

in variability of the F_2 over the F_1 is demonstrated by the standard deviations and frequency histograms (figure 3). The significant difference between the two F_2's, 334 and 344, is not explained at present. A third F_2, 354, grown more recently, had a mean of 768 ± 8; and the standard deviation was 156 ± 6. This mean is still larger than that of 344, but it is interesting to note that it is almost exactly halfway between the means of *Langsdorffii*

and *Sanderae*. Perhaps the shifting F_2 means are caused by a nearer approach to homozygosity in the parents used.

In table 1 are listed, in addition, the means and standard deviations of four F_3's, four F_4's, and reciprocal backcrosses of the F_1 to the original parents. The reciprocal backcrosses to *Langsdorffii* are equal in magnitude and intermediate in size and variability between the F_1 and *Langsdorffii*. The reciprocal backcrosses to *N. Sanderae* are also approximately equal in magnitude and are intermediate in size and in variability between the F_1 and Sutton's Scarlet.

TABLE 1

Statistical constants of the size index in the parent species and in hybrid generations.

FAMILY NUMBER	PARENTAGE	n	DESIGNATION	MEAN	S.D.
341	33043–24 selfed	47	*N. Sanderae*	1292± 5	48± 3
330642, 3327, 342		62	*N. Langsdorffii*	37± 3	32± 2
33046, 3327, 3401		78	F1 (*L.*×*S.*)	742± 4	46± 3
334	33046–19 selfed	139	F2 (*L.*×*S.*)	568± 7	125± 5
344	3401–3 & 5	238	F2 (*L.*×*S.*)	729± 6	136± 4
346	3401–3×33043–24	24	F1×*Sanderae*	1045±14	99±10
347	33043–24×3401–3	91	*Sanderae*×F1	1085± 5	72± 4
336	33046–19×330642–1	159	F1×*Langs.*	317± 5	90± 3
335	330642–1×33046–19	120	*Langs.*×F1	312± 7	107± 5
3402	334–3 selfed	28	F3 (528)	489±10	78± 7
34018	334–24 selfed	28	F3 (401)	448± 6	44± 4
3425	334–7 selfed	60	F3 (671)	547± 6	63± 4
3426	334–100 selfed	52	F3 (701)	699± 9	101± 7
348	3402–3 selfed	26	F4 (548)	408± 5	39± 4
349	3402–11 selfed	31	F4 (431)	384± 6	52± 4
3411	3402–28 selfed	104	F4 (660)	578± 4	53± 2
3412	34018–18 selfed	95	F4 (413)	337± 5	72± 4

Individual plants from various points on the F_2 frequency curve gave F_3 populations differing markedly in their means and variability. No F_3 family was as variable as the F_2. With one exception, all F_4 populations were less variable than the F_3 populations from which they came. With a single exception, the means of the F_2, F_3, and F_4 families were lower than the means of the F_1, F_2, and F_3 families from which they were derived, irrespective of whether the parent plant was below or above the mean of its family. The size of the F_2 and F_3 parent plants is listed in table 1 in parentheses after the designation of the F_3 and F_4 families to which they gave rise.

LINKAGE BETWEEN QUALITATIVE AND QUANTITATIVE CHARACTERS

Association between color and size in the F_2 and first backcross generations

Since the color genes contributed by each parent are known (SMITH 1937), the average size of flowers with a certain gene from the larger parent

may be calculated and compared with the average size of those having the corresponding allele (or alleles) from the smaller parent. The difference between the two averages is considered to represent the contribution to size of the quantitative factors which are linked with each color gene. The results of such a series of calculations in two F_2 families, 334 and 344, are listed in table 2. The most outstanding result is that every color gene from the larger parent is associated with larger-sized corollas. Most of the differences are statistically significant. In those cases which are questionable, the consistency of the results in this and in other groups of populations leaves no room for doubt that they are biologically significant.

TABLE 2

Summary of linkages between color characters and corolla size in the F2 generation.

COLOR FACTORS	FAMILY 334 n=139			FAMILY 344 n=238		
	MEAN	DIFFERENCE	DIFF./PE	MEAN	DIFFERENCE	DIFF./PE
pp	484			667		
P	595	+111	7.9	744	+77	3.9
very light	515			620		
light	537	+22	1.2	695	+75	4.4
intense	634	+97	5.7	764	+69	6.6
very intense	676	+42	2.1	834	+70	5.3
rr	568			749		
R	667	+99	5.2	762	+13	0.9
e1e1e2e2	556			703		
E1, E2, E1E2	616	+60	3.5	748	+45	2.1
G	544			714		
gg	627	+83	5.9	835	+121	8.6
B1B2	559			699		
white	640	+81	4.8	796	+97	8.8

The average effect for each group of genes in the two F_2's has been calculated (without weighting for differences in number) and listed in the first column in table 4. Each value obtained is an approximation of the size contribution of the chromosome in which that particular gene is located. Since the data can not be refined at present to make allowances for crossing over, the figures probably underestimate each chromosomal contribution. For means of comparison, if each one of the nine chromosomes added equally to the difference in size between the two original parents, their contribution would be 140 size units apiece. All the calculated values values are less than this. There is no indication from these results nor from those in subsequent generations that any gene or group of genes has an outstanding large effect on size. A number of genes with comparatively minor effects probably are concerned.

Some of the genes for small size are associated with dominant color characters, so that smallness cannot be attributed to an accumulation of recessive genes other than those which visibly influence only size.

Within the group having green plastid color (*G*), the various degrees of greenness, namely green, yellow and cream, were found to be associated with size differences. Similarly, within the group which have blue pollen color (*B1B2*), the various degrees of blueness also were found to be associated with size differences.

Backcrosses of the F_1 to *N.Langsdorffii* were useful only for an analysis of the size effect in *pp* versus *Pp* individuals. These results are listed below:

Family	*n*	*pp*	*Pp*	*Difference*	*Diff./P.E.*
335	120	280	338	+58	4.1
336	159	294	334	+40	3.4
345	31	312	384	+72	3.1

Families 335 and 336 are directly comparable with the F_2 family 334. The difference in size between the means of *pp* and *Pp* is roughly half that of the difference between *pp* and (*PP*+*Pp*).

The backcrosses of the F_1 to *N. Sanderae* could be used only for an analysis of pollen-color genes and size. The results were as follows:

Family	*n*	*B1b1B2b2*	*White*	*Difference*	*Diff./P.E.*
338	74	909	963	+54	6.0
346	23	1001	1045	+44	0.5

Family 338 is derived from the same F_1 plant as F_2, 334. The effect of *B1B2* in the heterozygous condition is less than when either one or both pairs are homozygous, as in the F_2. Family 346 compared with 344 gave similar results. There appears to be good evidence that the size genes associated with *P* and with pollen color have less effect in the heterozygous than in the homozygous condition.

The reciprocal backcrosses of the F_1 to *N. Sanderae* offer material for detecting size differences associated with the sterility alleles. Under the discussion of pollen color (SMITH 1937), genetic evidence was presented which demonstrated that the male gametes with the *Sf* allele from *Langsdorffii* were eliminated in the cross *Sanderae*×F_1. If genes for small size are linked with *Sf*, the population resulting from the cross F_1×*Sanderae* should be smaller than the reciprocal cross. Compare the means of family 346 and 347 in table 1. The difference between the two is 40 ± 14.9. This result is in the predicted direction, but can not be considered statistically significant. The numbers are small, especially in 346. The reciprocal backcrosses 337 (*Sanderae*×F_1) and 338 (F_1×*Sanderae*) were not listed in the table because they were not derived from identically the same plants. 337 (33043–24×33046–9) has a mean of 1090 ± 6. The mean of 338 (33046–19×33043–19) is 952 ± 5, which obviously is a significant difference. It

was concluded that size genes are linked with the self-sterility locus. This result was predictable, since pollen color was found to be associated with size differences, and a gene for blue pollen is located in the same chromosome as the *s* alleles.

Association of color and size in subsequent generations

One of the most successful methods for consistently testing pronounced linkages between color and size, was to cross with *Sanderae* various small segregants with many recessives. The resulting F_1's were selfed and crossed back to the small parent. Segregating populations from these types of crosses were obtained including four families from self-pollinations, namely: 3503, 3504, 3509, and 35011; and three families from backcrosses, namely:

TABLE 3

Association between color and size in families obtained from crossing various small segregants with Sanderae, then selfing the F1 or backcrossing it to the small segregant.

FAMILY NUMBER	DIFFERENCE BETWEEN MEAN SIZES OF SEGREGATING COLOR ALLELES				
	P-p	*R-r*	*E-e*	*g-G*	*white-B1B2*
3503	+61±28	+113±36	+121±28		+239±19
3504	+15±17	+20±20			+64±17
3509		+53±13			
35010				+70±42	
35011	+3±25	+138±26	+93±32		+141±26
35012	+48±17	+38±39	+102±26		
35013			+85±19		
Average	31.8	72.4	100.3	70.0	144.3

35010, 35012, and 35013. The average flower size associated with color genes in 35011 is illustrated in plate 1. A summary of the data on all these families is shown in table 3. The size genes associated with intensity of anthocyanin color are omitted in the table and will not be discussed further because of the difficulty in classifying and analyzing intensity. Nevertheless, the association was usually quite pronounced; for example, in family 35021 which is four generations removed from the original parents, the average size of *intense* (*I1i1i2i2*) segregants was 490±7, while that of *very intense* (*I1I1I2i2*) segregants was 512±15.

The average value for each color gene in the families in table 3 is listed in the third column of figures in table 4. These values are of interest because they should give a better estimate than F_2 data of the size contributed by genes close to each color gene. The increased size value over that in the F_2 of three of the genes, namely *R*, *b1b2*, and *E1E2* was unexpected. More data are needed before a satisfactory explanation can be offered.

PLATE I

Family 35011: segregation of color and size. Each flower illustrated is representative of a color class and is as near as possible to the mean size of that group. Means are expressed in size index units, 1000 (log tube length+log lobe length), below the representative flower for each color class. About $\frac{1}{2}$ natural size.

pp = 729 ± 22
P = 732 ± 13
rr = 655 ± 19
R = 793 ± 17
Light = 701 ± 15
Intense = 749 ± 27
Very Intense = 822 ± 22
ee = 675 ± 16
E = 768 ± 15
B_1B_2 = 716 ± 11
White = 857 ± 23

TABLE 4

Average size values associated with color genes in different types of populations. Size is expressed as the difference between two means.

COLOR GENES	F_2 *Langs.*×*Sand.*	F_3 *Langs.*×*Sand.*	F_1 (SEG.×*Sand.*) ×SELF AND SEG.
P	+94.0	−3.0	+31.8
R	+56.0	+57.0	+72.4
E1E2	+52.5	+46.5	+100.3
g	+102.0	+86.0	+70.0
b1b2	+89.0	+71.3	+144.3

Linkages between color and size genes in some F_3 families are tabulated as averages in table 4. In each case, with the exception of size associated with the *R* factor, there is a marked numerical decrease from the F_2 values. This is to be expected if crossing over takes place; and is, therefore, a strong point favoring the interpretation that we are dealing here with separate genes affecting size rather than the manifold effect of genes which influence

TABLE 5

Five cumulative series made from F2 data showing increased size with each single addition of a color character from the large parent.

FACTORIAL SERIES	FAMILY 334 n	FAMILY 334 MEAN	FAMILY 334 DIFF.	FAMILY 344 n	FAMILY 344 MEAN	FAMILY 344 DIFF.
1.						
pGB1B2	18	466		9	556	
PGB1B1	57	556	+90	91	694	+138
PgB1B2	21	651	+95	18	775	+81
Pg white	10	687	+36	15	900	+125
2.						
rGB1B2	12	484		20	693	
rG white	3	615	+131	15	776	+83
RG white	12	640	+25	40	791	+15
Rg white	7	696	+56	12	881	+90
3.						
eGB1B2	26	525		14	660	
EGB1B2	30	585	+60	77	701	+41
EG white	12	566	−19	62	776	+75
Eg white	9	667	+101	16	874	+98
4.						
reG	6	462		2	690	
rEG	8	555	+93	38	727	+37
REG	31	631	+76	100	750	+23
REg	15	689	+58	23	845	+95
5.						
reB1B2	8	483		2	690	
rEB1B2	11	579	+96	19	708	+18
rE white	4	613	+34	20	790	+82
RE white	16	661	+49	50	816	+26

both color and size. Another point supporting this argument is that in every segregating population it is possible to find some plants with large corollas but many qualitative characters from the small parent, and others with small corollas and many color factors from the larger parent. It is often not possible to determine which genes are associated with the larger size until the averages are calculated. In other words, crossovers are frequent.

TABLE 6

Increase in size by adding single genes or groups of genes in an accumulating series.

FAMILY 3503				FAMILY 3504			
COLOR FACTORS	n	MEAN	DIFF.	COLOR FACTORS	n	MEAN	DIFF.
PrB1B2	1	723		*PrB1B2*	4	933	
PRB1B2	6	919	+196	*Pr White*	5	983	+50
PR White	10	1045	+126	*PR White*	12	1012	+29
pB1B2	2	926		*pB1B2*	5	970	
p white	3	980	+54	*PB1B2*	10	935	−35
P white	13	1017	+37	*P white*	17	1015	+80
Pri2B1B2	1	723		*Pri2B1B2*	2	909	
PRi2B1B2	2	880	+157	*PRi2B1B2*	4	945	+34
PRi2 white	4	1043	+163	*PRI2B1B2*	3	910	−35
PRI2 white	6	1046	+3	*PRI2 white*	10	1049	+139
FAMILY 35011				FAMILY 35012			
COLOR FACTORS	n	MEAN	DIFF.	COLOR FACTORS	n	MEAN	DIFF.
pB1B2	10	698		*Pre*	4	455	
PB1B2	30	720	+22	*PrE*	2	515	+60
P white	3	842	+122	*PRE*	7	530	+15
PreB1B2	1	645		*ei1i2*	4	394	
PReB1B2	7	716	+71	*e* all other intensities	4	455	+61
PREB1B2	12	783	+67	*E* all other intensities	9	527	+72
PRE white	3	842	+59				

Cumulative effect of size genes

So far we have seen that every color gene considered in these experiments is associated with size differences. If a series were made up in which each successive member contains one more qualitative gene from the larger parent, then the average size should increase with each additional group as the series progresses if the genes are cumulative. Five representative series were constructed from the F_2 data of families 334 and 344. They are arranged in table 5.

The results show definitely that each addition of a color gene and its associated size gene or group of genes from the larger parent causes an increase in corolla size. The genes are cumulative in effect.

Similar series were also constructed from the data on families 3503, 3504, 35011, and 35012 (table 6). These results, with two exceptions, were all in the expected direction. The classes are regrettably small in number.

INTERACTION OF SIZE GENES

The next step, after demonstrating that size genes are cumulative in effect, is to investigate the problem of the manner in which their values accumulate. The approximately symmetrical nature of the F_2 frequency distribution curve, after the simple and logical transformation $x^1 = \log x$, indicated that the variability is due to a *large* number of *independent, additive* factors with effects of *comparable magnitude.*

The following additional considerations support the conclusion that a large number of genes affect corolla size. (1) Neither of the two extremes of the F_2 distribution reached the parental classes. (2) The F_2 curve is not definitely bi- or multimodal. (3) By using the formula for estimating the number of size genes it was found that *not less* than twelve were involved. (4) Each color gene was associated with comparatively small size effects. There was no indication that genes with major effects on corolla size were operating. (5) Furthermore, if there were a few genes with effects comparable to all the others put together (or greater), there is likely to be marked negative skewness in the F_2 population.

The size genes are independent because they have been found to be linked with independent color genes. The results in table 2 indicate that they are probably of comparable magnitude.

If genes interact so that the visible effect of each gene is smaller the greater the number of genes acting in the same direction (RASMUSSON 1933), then the F_2 distribution curve will be negatively skewed. In other words, after an appropriate scale has been chosen (in this experiment a logarithmic one) any departure from a normal curve in the direction of negative skewness may indicate interactions of the type described above. From the symmetrical nature of the F_2 curve, we are led to conclude that, in a general way, there is no indication that the genes interact. They appear to be additive in the sense that they add independent geometric (logarithmic) increments of size. The situation might be still more complex; for example, interactions of the RASMUSSON type might occur but not be detectable on the scale used here because some size genes contributed increments exceeding regular geometric values.

If each size gene or group of genes which is associated with a certain color gene is entirely unaffected by the size genes associated with another color gene, then the difference in size between R and r, for example, should be the same in the P as in the p universe. Any significant departure from this type of result may indicate an interaction. Fourfold tables were con-

structed for each possible group of two gene combinations in the F_2. Only two groups, *R–g*, and *E1E2–g*, gave consistent evidence of interactions in F_2's 334 and 344, and these were of the type predicted by RASMUSSON. However, neither of these groups showed evidence of an interaction in a more recently calculated F_2, 354. In conclusion, there were no consistent results showing definitely an interaction between the size genes associated with any two color characters.

SUMMARY

Jn populations derived from the cross *Nicotiana Langsdorffii*×*N. Sanderae*, each of the color genes studied was found to be linked with genes affecting corolla size. There were apparently a large number of size genes involved, and these had effects of comparable magnitude. None with major effects was detected. Some, if not all, are non-dominant in the sense that Aa has less effect than AA. The size genes were proved to act in a cumulative manner. There was no consistent indication of any interaction between the size genes linked with any two color genes. Linkage was found between the self-sterility locus and size genes.

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GENETICS OF PLATYPOECILUS

III. INHERITANCE OF SEX AND CROSSING OVER OF THE SEX CHROMOSOMES IN THE PLATYFISH[1]

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INTRODUCTION

GENETIC evidence of crossing over between the sex chromosomes of heterogametic individuals (XY males and WZ females) is so rare in animals as to give special significance to additional data on this occurrence.

AIDA (1921) has shown in the Japanese ricefield killifish, Aplocheilus, that the X and Y chromosomes of the male occasionally cross over. In 1930 he presented additional data on this, together with some interesting data involving aberrant sex ratios, which he interpreted as due to nondisjunction. In 1936 AIDA re-interpreted the abnormal sex ratios as due to sex reversal, following the suggestions of WITSCHI and WINGE.

WINGE (1923, 1927) has shown in the West Indian guppy, Lebistes, that the X and Y occasionally cross over. In 1930 he stated that the genotypic chromosomal constitution of XX for femaleness in this fish may be overruled by other sex-determining factors so that the XX complex may occasionally produce a male. These XX males when mated to normal XX females produce, as might be expected, 100 percent daughters. This work he confirmed and extended in his papers of 1932 and 1934.

FRASER and GORDON (1928, 1929) presented the first genetical evidence for crossing over between the W and Z chromosomes of a female Mexican platyfish (*Platypoecilus maculatus*). BELLAMY (1933) accepted this interpretation and added several cases of his own. The data to be described in this paper increase the number of possible cases of crossing over between the W and Z from two to seven. A possible case (the first to be recorded) of crossing over between the two Z chromosomes of the male platyfish is also described.

The new experiments are concerned chiefly with an analysis of the inheritance of a black banded pattern and its relation to other patterns. The variety with this pattern is known as the black platy. The symbol *N* ('nigra' or 'niger') was given to it by BELLAMY (1922). The history of domestication of this and other varieties of platyfish has been given by GORDON

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(1934). BELLAMY (1922, 1924, 1928, 1933) showed that the black pattern (*N*) is inherited as a sex-linked dominant which follows the WZ type of sex inheritance. The data of the present paper confirm this.

BELLAMY also demonstrated that the 'rubra' pattern, a red body color with many large black spots, behaves as dominant and sex-linked. In BELLAMY'S earlier papers *R* represented the complete 'rubra' pattern: *R* represented not only the red body color in the 'rubra' platyfish but large black spots (macromelanophores) as well. Since BELLAMY listed still another factor, *P*, for large black spots, GORDON failed to appreciate the point that *R* was supposed to have a dual effect in influencing the development of large black spots and red body color. It appeared (GORDON 1927) that 'rubra' contained two genes: *R* (red body color), and *Sp* (large black spots). *Sp* is now thought to be synonymous with *P* ('pulchra' pattern).

In 1929 FRASER AND GORDON showed that 'rubra' is actually a *double* dominant type and BELLAMY (1933) has accepted this interpretation. The early formula of BELLAMY for the 'rubra' male was *RR* and for the female *RO* where *O* represents the heterochromosome. Gordon has used *R Sp R Sp* for male 'rubra' and *R Sp r sp* for female 'rubra' for the same genotypes. With the development of a strain of 'rubra' females that yielded mother-to-daughter type of inheritance, the practice was adopted of associating the genes with particular sex chromosomes; so that a female 'rubra' may have either one of the three genotypes: (Z) *R Sp* (W)*r sp*, (Z)*R Sp* (W)*R Sp* or (Z)*r sp* (W)*R Sp*. The behavior of these types was described by FRASER and GORDON (1929).[2]

EXPERIMENTAL DATA

The technique of handling and breeding tropical viviparous fishes has been described elsewhere (Gordon 1926, 1932).

Since no confirmatory data have been published on the sex-linked in-

[2] A comparison of terms used by BELLAMY and by GORDON is given below:

BELLAMY		GORDON	
COLOR VARIETY	SYMBOL	SYMBOL	COLOR VARIETY
Nigra	*N*	*N*	Black
Pulchra	*P*	*Sp*	Spotted
Rubra	*R*	*R* and *Sp*	Spotted, red
		R	Red (pure)
Recessive to all the dominant sex-linked factors above are:			
White	*W*	*n r sp*, *St*	Stippled
Wild	+	*n r sp*, *St*	Stippled
Gold	*g*	*n r sp*, *st*	Gold

The stippled platy carries the autosomal gene *St* which is dominant over gold, *st*. This was demonstrated by GORDON (1927) and confirmed by BELLAMY (1933). The discovery and subsequent history of the gold platyfish was presented by GORDON (1935).

heritance of the black pattern *N*, some preliminary work on this will be presented. Some of these crosses were made as early as 1925. In matings 1 and 2 (table 1) where both parents were black, the results indicate that the original stocks were true-breeding. In each case only the numbers of fishes which have survived to the sex-differentiating stage are given in

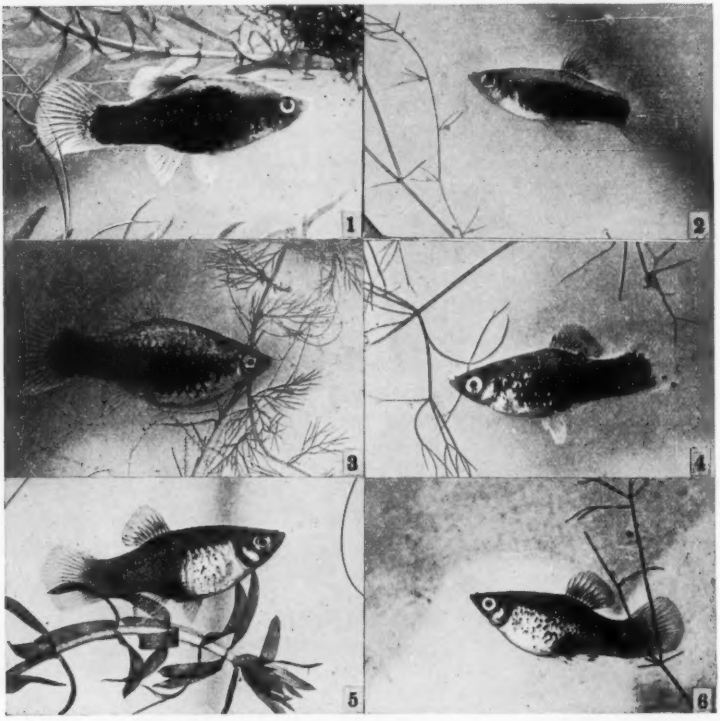

FIGURE 1.—The platyfish of Southern Mexico, *Platypoecilus maculatus*. Females on the left, males on the right.

The pair of fish on the top row are black platyfish.

The middle row shows a pair of black, red and spotted platyfish—trihybrids between the blacks above and the red and spotted below.

The bottom pair are red, spotted platyfish, the 'rubras.'

the tables. In most cases the figures given in the tables represent only a fraction of the total brood. The number of deaths before sexual differentiation sometimes is considerable. The period before maturity varies from six to ten months.

The first indication of sex linkage may be seen in the results of matings 5 and 6 which involved a black female and a non-black male. All of the

black progeny, except one, became males while all the non-black offspring became females. The exceptional black female may be explained as due to crossing over between the W and Z chromosomes of the female parent in the same fashion as previously outlined for the *Sp* gene (FRASER and GORDON 1928, 1929). Unfortunately it was impossible to confirm this assumption because of the sterility of the exceptional female.

In two other matings, where a female black platy was mated to a non-black male (matings 3 and 4) two phenotypes again appeared. The extraordinary feature of the two broods produced is that they are *all males*. These aberrant sex ratios will be discussed later.

When a heterozygous black male is mated with a recessive female, two phenotypes again appear but both sexes are represented in each group. These results are listed under crosses 7 and 8. This supports the idea that the male platyfish is homogametic.

A somewhat similar cross to those of 7 and 8 may be seen under mating number 9. In this case the female was a red platy heterozygous for red with the dominant *R* gene in the Z chromosome while the male was heterozygous for black: (W)*n r* (Z)*n R*×(Z)*N r* (Z)*n r*. The results are in accordance with theory, for among the offspring the red fishes, whether black or non-black, are all males; all the non-red fishes are females. Furthermore, among the black fishes, either in the red or non-red group, there were both males and females.

Mating number 9 was made to get double dominant males for backcross experiments to detect linkage relations. Double dominant males *N R* were obtained. When backcrossed to recessive females *n r* they gave no crossovers (mating 10). This indicates that *N* and *R* are either closely linked, or as Bellamy suggests, allelic.

Triple dominant males

Triple dominant males were obtained by mating *R Sp* females with *N* males—matings 11, 12 and 13. The *R* and *Sp* genes were carried by the Z chromosome of the mother, (Z)*n R Sp* (W)*n r sp*, and were transmitted to all of her sons. Half of these sons also received *N* from their heterozygamic father, (Z)*N r sp* (Z)*n r sp*, and consequently twenty triple dominant males were obtained, presumably (Z)*n R Sp* (Z)*N r sp*.

In mating a brother (Z)*N r sp* (Z)*n R Sp* and a sister (Z) *N r sp* (W)*n r sp* of brood number 151 (from mating 13), no unusual ratio appeared. This is indicated under mating 14.

Backcross of triple dominant males

In a series of matings (15, 16, and 17) an attempt was made to get large numbers of progeny by backcrossing triple dominant males, (Z)*N r sp* (Z)*n R Sp* to recessive females (Z)*n r sp* (W)*n r sp*.

TABLE 1

MATING NUMBER	FEMALES		MALES		PROGENY				
	PEDIGREE NUMBER	GENETIC FORMULA	PEDIGREE NUMBER	GENETIC FORMULA	FED. OF YOUNG	FEMALE		MALE		CROSS OVERS
						(W)*n* (Z)*N*	(W)*n* (Z)*n*	(Z)*N* (Z)*N*	(Z)*n* (Z)*n*	
1.	64a	(W)*n* (Z)*N*	64b	(Z)*N* (Z)*N*	64	14	0	13	0	
2.	64–3	ditto	64–11	ditto	87	15	0	14	0	
						(W) (Z)	(W) (Z)	(Z)*N* (Z)*n*	(Z)*n* (Z)*n*	
3.	64c	(W)?*n* (Z)?*N*	?	(Z)*n* (Z)*n*	65	0	0	7	7	
4.	64–4	W)?*n* (Z)?*N*	43–12	(Z)*n* (Z)*n*	89	0	0	25	21	
						(W)*n* (Z)*N*	(W)*n* (Z)*n*	(Z)*N* (Z)*n*	(Z)*n* (Z)*n*	(W)*N* (Z)*n*
5.	88–1	(W)*n* (Z)*N*	40–12	(Z)*n* (Z)*n*	116	0	12	11	0	1*
6.	88–2	ditto	121–11	ditto	150	0	42	31	0	0
						(W)*n* (Z)*N*	(W)*n* (Z)*n*	(Z)*N* (Z)*n*	(Z)*n* (Z)*n*	
7.	43–8	(W)*n* (Z)*n*	65–11	(Z)*N* (Z)*n*	88	3	5	4	3	
8.	66–1	ditto	88–11	ditto	105	8	14	12	11	
						(W)*nrsp* (Z)*Nrsp*	(W)*nrsp* (Z)*nrsp*	(Z)*nRsp* (Z)*nrsp*	(Z)*nRsp* (Z)*Nrsp*	
9.	60–4	(W)*nrsp* (Z)*nRsp*	105–11	(Z)*Nrsp* (Z)*nrsp*	142	17	25	24	19	
						(W)*nrsp* (Z)*Nrsp*	(W)*nrsp* (Z)*nRsp*	(Z)*nrsp* (Z)*nRsp*	(Z)*nrsp* (Z)*Nrsp*	
10.	142–1, 2	(W)*nrsp* (Z)*nrsp*	142–11, 12	(Z)*Nrsp* (Z)*nRsp*	146	17	14	20	15	
						(W)*nrsp* (Z)*nrsp*	(W)*nrsp* (Z)*Nrsp*	(Z)*nRsp* (Z)*nrsp*	(Z)*nRSp* (Z)*Nrsp*	
11.	40–4	(W)*nrsp* (Z)*nRSp*	89–12	(Z)*Nrsp* (Z)*nrsp*	149	5	2	4	2	
12.	108–1	ditto	89–13	ditto	151	12	14	13	14	
13.	40–5	ditto	105–31	ditto	140	9	8	7	4	
						(W)*nrsp* (Z)*nRSp*	(W)*nrsp* (Z)*Nrsp*	(Z)*Nrsp* (Z)*Nrsp*	(Z)*nRSp* (Z)*Nrsp*	
14.	151–32	(W)*nrsp* (Z)*Nrsp*	151–11	(Z)*nRSp* (Z)*Nrsp*	156	13	14	13	13	
						(W)*nrsp* (Z)*Nrsp*	(W)*nrsp* (Z)*nRSp*	(Z)*nrsp* (Z)*Nrsp*	(Z)*nrsp* (Z)*RSp*	(W)*nrsp* (Z)*nRsp*
15.	52–5	(W)*nrsp* (Z)*nrsp*	149–11	(Z)*nRSp* (Z)*Nrsp*	153	15	19	15	19	0
16.	83–1	ditto	149–11	ditto	154	8	11	9	11	0
17.	151–2	ditto	149–12	ditto	155	28	33	28	34	1*

TABLE 1 (*Continued*)

MATING NUMBER	FEMALES: PEDIGREE NUMBER	FEMALES: GENETIC FORMULA	MALES: PEDIGREE NUMBER	MALES: GENETIC FORMULA	PED. OF YOUNG	PROGENY: FEMALE		MALE		CROSS OVERS
						(W)*nrsp* (Z)*nrsp*		(Z)*nRsp* (Z)*nrsp*		
18.	155–1	(W)*nrsp* (Z)*nRsp*	148–33	(Z)*nrsp* (Z)*nrsp*	166	19		21		
						(W)*nRSp* (Z)*nRSp*	(W)*nRSp* (Z)*Nrsp*	(Z)*nrsp* (Z)*nRSp*	(Z)*nrsp* (Z)*Nrsp*	(Z)*nRSp* (Z)*Nrsp*
19.	136–1	(W)*nRSp* (Z)*nrsp*	151–11	(Z)*nRSp* (Z)*Nrsp*	157	11	12	13	12	1*
						(W)*nrsp* (Z)*nRSp*	(W)*nrsp* (Z)*Nrsp*	(Z)*nrsp* (Z)*nRSp*	(Z)*nrsp* (Z)*Nrsp*	
20.	140–1	(W)*nrsp* (Z)*nrsp*	157–11*	(Z)*nRSp* (Z)*Nrsp*	168	15	12	12	15	
21.	148–1	ditto	157–11*	ditto	169	9	8	9	5	
						(W)*nRSp* (Z)*nrsp*	(W)*nRSp* (Z)*Nrsp*	(Z)*nrsp* (Z)*nrsp*	(Z)*nrsp* (Z)*Nrsp*	(Z)*nRSp* (Z)*nrsp*
22.	175–1	(W)*nRSp* (Z)*nrsp*	171–11	(Z)*nrsp* (Z)*Nrsp*	191	7	6	8	7	1*
						(W)*nRSp* (Z)*nrsp*	(W)*nRSp* (Z)*Nrsp*	(Z)*nrsp* (Z)*nrsp*	(Z)*nrsp* (Z)*Nrsp*	
23.	157–1	(W)*nRSp* (Z)*Nrsp*	148–32	(Z)*nrsp* (Z)*nrsp*	165	25	0	0	28	
24.	191–1, 2	ditto	175–16	ditto	205	16	0	0	20	
25.	170a	ditto?	175–16	ditto	170	2	0	0	2	
26.	171a	ditto?	?	(Z)*Nrsp* (Z)*nrsp*	171	7	9	0	19†	
27.	171a	ditto?	175–15	(Z)*nrsp* (Z)*nrsp*	171b	22	0	0	19	
						(W)*RSp* (Z)*rsp*	—	—	(Z)*rsp* (Z)*rsp*	(Z)*RSp* (Z)*rsp*
28.	165–4	(W)*RSp* (Z)*rsp*	161–12	(Z)*rsp* (Z)*rsp*	175	18	—	—	15	1*
29.	165–6	ditto	161–12	ditto	176	19	—	—	17	1*
						(W)*rsp* (Z)*RSp*	(W)*rsp* (Z)*rsp*	(Z)*rsp* (Z)*RSp*	(Z)*rsp* (Z)*rsp*	
30.	148–9	(W)*rsp* (Z)*rsp*	191–11*	(Z)*RSp* (Z)*rsp*	198	5	4	6	5	
31.	151–1	ditto	175–19*	ditto	193a	4	5	3	4	
32.	151–2	ditto	176–18*	ditto	193b	7	6	8	9	

* Refers to exceptions—the crossover types.

† This group of 19 includes fishes of two genotypes: NN and Nn.

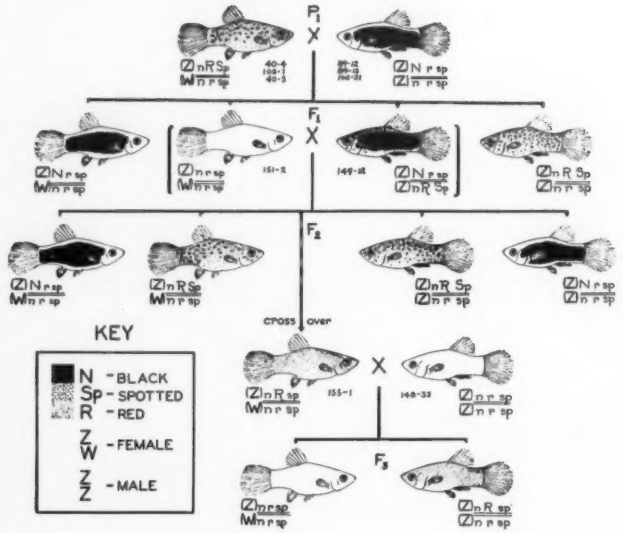

FIGURE 2.—Crossing over of the two Z chromosomes.

The parental fish represent those used in matings 13, 14 and 15 and the first generation fishes are of four genotypes as indicated. Note the genotype of the red, spotted female. Its *R* and *Sp* factors are carried on the Z chromosome.

A triple recessive gold female (151–2) and a triple dominant black, red and spotted male (149–12) are selected as breeders for the second generation.

They produced in the second generation one exceptional type, the red, non-spotted female (155-1) indicated by the arrow with a black and white shaft. This fish appears in mating 17 of the table in the text.

The exceptional red, non-spotted female (155-1) is backcrossed to a gold male (148-33) with the typical criss-cross behavior characteristic of the WZ-ZZ type of inheritance. This exceptional red, non-spotted female (155-1) is the product of crossing over of the two Z chromosomes of its father (149-12). This is the first instance reported of crossing over of the Z chromosomes in this species.

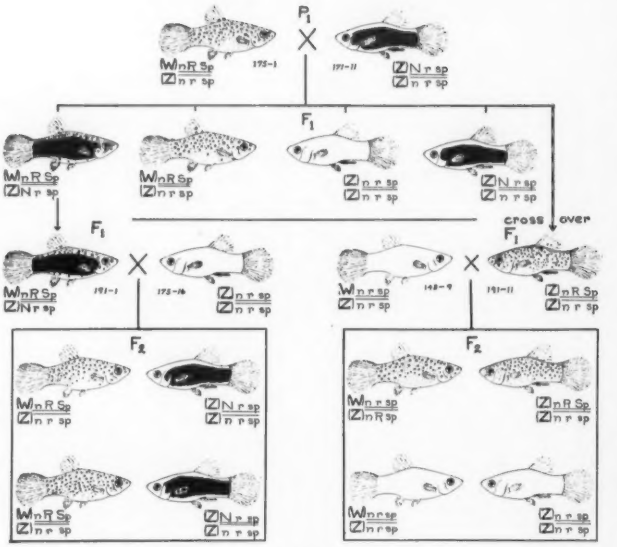

FIGURE 3.—Crossing over of the Z and W chromosomes.

The parental fish represent those used in mating 19. Note the genotype of the red, spotted female (175-11). The factors *R* and *Sp* are carried on the W chromosome.

The four types expected appear in the first generation—but an exceptional also appears, a red, spotted male (191-11) indicated by the arrow with the black and white shaft to the extreme right, third row.

The exceptional red, spotted male (191-11) when backcrossed to the double recessive gold female (148-9) gives a typical brood of red, spotted and non-red, non-spotted young.

The triple dominant female, that is black, red and spotted (191-1) is back crossed to a triple recessive gold male and it produces typical results: red, spotted daughters and black sons, reproducing the genetic types of their grandparents.

Out of 231 fishes which were reared to adult size there was but a single exception from the expected. The exceptional red, unspotted female, *R* (pedigree number 151-1) provides a clue to the possible arrangement of the three sex-linked genes in the Z chromosome. If it be assumed that this red female (Z)*n R sp* (W)*n r sp* was the result of a single crossover of the two Z chromosomes of the male then:

(1) *R* lies between *n* and *Sp*, or
(2) *n* lies between *R* and *Sp*.

In either case *R* lies to the left (or top) of *Sp* as shown by FRASER and GORDON (1929). Because of the closeness of *R* and *Sp* as indicated by relative infrequency of crossing over between them the locus of *n* is tentatively placed to the left of *R*. The gamete (Z)*n R sp* from the male, 149-12, resulted from crossing over of its chromosomes (Z)*N r sp* and (Z)*n R Sp*. The male contributing gamete (Z)*n R sp* joined with the recessive female contributing gamete (W)*n r sp* to produce the exceptional red daughter (Z)*n R sp* (W)*n r sp*. The fact that the exceptional female (155-1) was actually of the above constitution was proved by mating it to a recessive male (mating 18). It produced red males, (Z)*n R sp* (Z)*n r sp*, and non-red females only, (Z)*n r sp* (W)*n r sp* (fig. 2).

BELLAMY (1928) reported what appears to be a similar backcross of triple dominant males to recessive females in his crosses 217.2, 255 and 290. He calls the males *NR* and females *WO*. It is now fairly certain that his *R* is *R* plus *Sp*, his *W* is a multiple recessive symbol and his *O* is a symbol representing the lack of dominance in the heterochromosome of the female. Therefore his males were phenotypically *N R Sp* and females *n r sp*. In the backcross he obtained 209 young: 41 black males (Z)*N r sp* (Z)*n r sp*, 51 black females (Z)*N r sp* (W)*n r sp*, 49 red, black spotted males (Z)*n R Sp* (W)*n r sp* and 68 red, black spotted females (Z)*n R Sp* (W)*n r sp*. The formulas given here are based on GORDON'S system. BELLAMY used the four formulas as follows: *NW*, *NO*, *RW* and *RO*. No crossovers were detected by BELLAMY in this series of experiments. While he does present three cases of crossing over between the W and Z chromosome of the female, elsewhere, none are presented of crossing over of the two Z chromosomes of the male. The one individual case of crossing over between the two Z chromosomes given in this paper is the only one yet reported. In two additional matings, 20 and 21, a triple dominant male (obtained as a crossover from mating 19, to be described below) was crossed to a recessive female but no crossover individuals appeared.

Crossing over between the Z and W chromosomes of the female is rare. About one per cent is the crude estimate of FRASER AND GORDON (1929), BELLAMY confirms this (1933) by his estimated value of about 0.8 percent.

Apparently crossing over of the two Z chromosomes of the male is rarer still.

Triple dominant females

FRASER and GORDON (1929) showed that the dominant genes *R* and *Sp*, which are usually found in the Z chromosome in most stocks of platyfish, may occasionally cross over and subsequently be carried by the W. These new females give mother-to-daughter type of inheritance.

Females which carried *R* and *Sp* on the *W* chromosome were mated to males carrying *N*. In the first cross of this series the male was triple dominant (mating 19). It was expected that one quarter of the offspring would be triple dominant females. The triple dominant females were obtained; but there was one triple dominant male also. This male's appearance may be explained as due to crossing over between the W and Z chromosomes of the female parent, with the result that *R* and *Sp* were transferred from the W back to the Z. It might be supposed that as a result of crossing over of the two Z chromosomes of the male a gamete (Z)*N R Sp* was formed, but the breeding behavior of this exceptional male (matings 20 and 21) indicates that the former explanation is probably the correct one.

In another experiment (mating 22) sixteen triple dominant females were obtained by mating *R Sp* females (in which *R* and *Sp* were carried on the W chromosome) to black *N* males. Another crossover between the W and Z chromosomes appeared. It was a *R Sp* male, pedigree 191–11. This male, mated to a recessive female (148–9), produced equal numbers of *R Sp* male and female young. These are classified under mating 30.

When the triple dominant females, (Z)*N r sp* (W)*n R Sp* were backcrossed to multiple recessive males, (Z)*n r sp* (Z)*n r sp*, as indicated by matings 23 and 24, only the normal types appeared, namely, red, black spotted females (Z)*n r sp* (W)*n R Sp* and black males (Z)*N r sp* (Z)*n r sp*.

OCCURRENCE OF TRIPLE DOMINANT FEMALES IN COMMERCIAL STOCKS

At the Wm. Tricker Co., Saddle River, New Jersey, I detected a young female platyfish which seemed to me to be a triple dominant. It was kindly donated, and when mated with a recessive male (mating 25), it produced only four offspring which survived. The two females were *n R Sp*, while the males were *N r sp*.

At the New York Aquarium Mr. C. L. COATES kindly gave me another female fish which I suspected of being triple dominant. Mr. COATES had obtained the female from The Aquarium Stock Co. of New York, which Company in turn had received it from Germany. As soon as I obtained this apparently triple dominant female I placed it with a multiple recessive male. The female first produced three classes of young, which indicated

that it probably had already been mated to a black male previously. The details are listed under mating 26. The second brood (mating 27), in which the multiple recessive male sperms were probably effective, showed only *n R Sp* females and *N r sp* males. The parents of these fishes were actually triple dominant and probably (Z)*N r sp* (W)*n R Sp* in constitution. Subsequently the daughters of the triple dominant mothers showed the typical mother-to-daughter type of inheritance. This indicates that not only in this laboratory, but elsewhere, crossing over from the Z to the W chromosome takes place occasionally.

FURTHER CASES OF CROSSING OVER OF THE Z AND W CHROMOSOMES

Matings 28 and 29 are concerned with *R Sp* females in which the two dominant genes are carried on the W chromosomes. When these (W)*R Sp* (Z)*r sp* females were backcrossed to recessive males, (Z)*r sp* (Z)*r sp*, they produced 37 *R Sp* females, 32 *r sp* males and two exceptional *R Sp* males. These males have been tested and proved to be fertile. The sex ratios of their progeny indicate a normal ZZ chromosome constitution (matings 31 and 32). The two exceptional males may be explained by crossing over between the W and Z chromosomes of their mother in such a way that the dominant genes were transferred from the W to the Z.

From the above work, and the work elsewhere described by FRASER and GORDON (1929), all the crossover gametes detected have been those which received *dominant factors* as a result of the WZ crossing over process. In each case of crossing over there should have been a corresponding gamete that possessed the recessive alleles. Yet none was detected.

On the other hand, the four cases of crossing over which BELLAMY (1928, 1933) reported show just the reverse situation. Here the gametes, produced by crossing over between the W and Z chromosomes, carried the recessives. BELLAMY crossed a heterozygous red, black spotted male (Z)*R Sp* (Z)*r sp* with a heterozygous red, black spotted female (Z)*R Sp* (W)*r sp*. He obtained 12 red black spotted males (Z)*R Sp* (Z)*R Sp* and (Z)*R Sp* (Z)*r sp*, 16 red, black spotted females (Z)*R Sp* (W)*r sp*, 11 non-red, non-black spotted females (Z)*r sp* (W)*r sp* and three crossover types, 3 non-red non-black spotted males (Z)*r sp* (Z)*r sp*. It is supposed that crossing over took place between the (Z)*R Sp* and (W)*r sp* chromosomes of the female parent and the recessive genes *r sp* were transferred from the W to the Z. As BELLAMY states, if these actually are cases of crossing over they have occurred four times (he had an additional case) among 962 offspring from matings in which detection was possible.

DISCUSSION AND CONCLUSIONS

The data given above and the entire body of data of BELLAMY, GORDON, and FRASER and GORDON given elsewhere indicate clearly that *N* for black,

R for red body color and *Sp* for spots are dominant sex-linked genes which follow the ZW type of sex inheritance. From the evidence of ZW crossing over and the establishment of mother-to-daughter type of inheritance in (W)*R Sp* (Z)*r sp* females, it is clear that ZW represents the sex chromosome constitution of the female platyfish, *Platypoecilus maculatus*.

The crossover individual, a *red* non-spotted male (32-11) reported by FRASER and GORDON (1929) was the first concrete evidence against the idea that *R* for body color and *Sp* for spotting were due to a single gene *R* of BELLAMY. This red male (Z)*n R sp* (Z)*n r sp* was obtained by crossing over between (Z)*n R Sp* and (W)*n r sp* in the germ cells of its female parent, with the result that an (Z)*n R sp* gamete was produced. This gamete joined with a gamete (Z)*n r sp* of the male parent and yielded the red male mentioned. Under the multiple allelic interpretation, the formula of a red, spotted female would have to be (Z)*R* (W)*Sp or* (Z)*sp* (W)*R*. But experiments indicate that it is (Z)*R Sp* (W)*r sp*, or (Z)*R Sp* (W)*R Sp*, or (Z)*r sp* (W)*R Sp*. *R* and *Sp* are not allelic.

From the crossover case cited under mating 17 of this paper, where a red female (Z)*n R sp* (W)*n r sp* appeared as a result of (Z)*N r sp* crossing over with (Z)*n R Sp* with the production of a (Z)*n R sp* gamete, it is thought that *N* is not allelic with *Sp*. If *N* were allelic to *Sp* then there would have been a *R N* gamete produced rather than *n R sp*.

From the data available *R* and *Sp* are quite close to one another as indicated by one percent crossing over of Z and W, and less than half a percent crossing over of Z and Z. Tentatively, therefore, *N* is not placed between *R* and *Sp*, although perhaps in time it might be found to have its locus there. *N*, tentatively, is placed to the left of *R*. It cannot, from the data, be located to the right of *Sp* unless the exceptional red female was the product of a double crossover. This is extremely unlikely.

Recent work on sex-reversal in animals indicates that fishes are particularly susceptible to shifting forces that determine sex. Both in Lebistes and in Aplocheilus belonging to the same order as Platypoecilus, many cases of aberrant sex ratios have been traced to complete sex reversal. In Xiphophorus, sex is particularly unstable. In this swordtail killifish of southern Mexico cytological and genetical studies indicate that a large proportion of each brood is potentially bisexual. WITSCHI (1932) in his admirable review concludes that there is probably no other fish in which sex inversions have been observed more frequently. In an unpublished manuscript, "Fresh-water Fishes of Northeastern Mexico," HUBBS and GORDON will report a confirmation of the uniqueness of Xiphophorus with regard to its labile sex state. In its native environment GORDON found in 1930 a large population of swordtails in a small lake just outside the business district of Jalapa, State of Vera Cruz, Mexico. The females were relatively uniform

in size but there were definitely two size groups in what appeared to be males. The external secondary sexual character, the long sword or extended tail was fully developed in both size groups. In the group of small males the gonopodia were well differentiated but in the group of large males most had imperfect gonopodia. These modified anal fins are used normally as intromittent organs. In many large fishes the gonopodia were in various stages of degeneration. The chief indicative point of this degeneracy was the flabbiness and rayless nature of the fins. Upon rough dissection all the large swordtail 'males' showed extremely small or no gonads, male or female; whereas the small swordtail males showed large, firm, normal, fused testes. HUBBS and GORDON interpreted the large 'males' as transformed females. The only other species of fish in this lake is *Pseudoxiphophorus bimaculatus*, which is not closely related to Xiphophorus and with which it is not likely to hybridize. No platyfish inhabit this lake or river system. The swordtail population, just mentioned, is in a natural state, yet even here the sex equilibrium is decidedly unstable. BREIDER (1935) finds that male swordtails predominate in some strains of Xiphophorus while females predominate in others. He believes that there is a genetic basis for sex determination in the swordtail but it has not yet been definitely established.

In contrast to the sex instability of the swordtail, *P. maculatus* is a model to which geneticists might point with some assurance. Except for one or two cases, fairly even sex ratios are exhibited by the platyfish in the laboratory. A definite case of sex reversal has never been reported in this species although statements to that effect appear from time to time. Aquarists often report that 'a change in sex' occurred in this species. The sex ratios for the common platyfish as represented by random catches of *P. maculatus* in Mexico are as follows:

COLLECTOR	FEMALES	MALES	RATIO
1867 SUMICRAST	7	6	1:1.2
1904 MEEK	42	26	1:1.6
1932 GORDON	62	39	1:1.6

Too much credence cannot be given to the data since there are several factors favoring the listing of a greater number of females. The males are smaller and more difficult to catch. Large, immature undifferentiated individuals would usually be classified as females; immature males appear like females but they eventually transform from female-like forms to male.

The two broods of one hundred percent males listed under matings 3 and 4 may have been the result of complete sex reversal in the parent fishes. The true genetic constitution of these parents may have been ZZ, and yet these animals were physiologically females. When mated to normal males ZZ, only male offspring might be expected.

Both WINGE with Lebistes and AIDA with Aplocheilus have obtained XY females when XX is their normal constitution, and XX males when XY is the normal constitution of the male. Each has built up an hypothesis based on the relative strengths of male-female factors in the sex chromosomes and in the autosomes. Each has suggested that it is no longer a puzzle why there happens to be the XY type in Lebistes and Aplocheilus and WZ type of sex determination in Platypoecilus, since by selection they are able to isolate heterogametic females and homogametic males in Aplocheilus and in Lebistes. Their explanation implies that nature has completed the work of selection for Platypoecilus with its ZZ-ZW constitution and that at one time Platypoecilus either had XX-XY or XX-XX type of sex determination. Apparently nature has done the job well in the common platyfish, for unlike WINGE's and AIDA's XY females, the WZ females of Platypoecilus are extremely stable, whereas the man-selected strains of XY female Lebistes and Aplocheilus are extremely unstable.

Up to this point wherever the name Platypoecilus was used it referred to *Platypoecilus maculatus*. There are four species of Platypoecilus: *P. couchianus* (Girard), *P. xiphidium* Hubbs and Gordon, *P. variatus* Meek and *P. maculatus* Gunther. In recent papers by KOSSWIG and by BREIDER the male of *P. variatus* and *P. xiphidium* is regarded as the heterogametic sex (like Lebistes and Aplocheilus) and the female as the homogametic sex. These conclusions are drawn from hybrid studies; for instance, *Xiphophorus hellerii* was first crossed with *P. maculatus* and this hybrid was mated to *P. variatus*. The triple hybrid was then crossed back in a number of ways. The conclusion that *P. xiphidium* male is heterogametic is also drawn from data of the behavior of hybrid fishes. BELLAMY (1936) has accepted KOSSWIG's conclusion of the XY type of sex determination for *P. variatus* and has published data on hybrids between *P. variatus* and *P. maculatus* in support of it.

It is well to keep in mind that these conclusions are based upon intergeneric and interspecific crosses only. No data on crosses between the many varieties within *P. variatus* or within *P. xiphidium* are presented in support of these conclusions.

With reference to the taxonomic position of *P. variatus* and *P. xiphidium*, HUBBS and GORDON have definitely separated these species from each other and from *P. maculatus*. Their specific distinctness is evident from a study of more than a thousand specimens. This distinctness becomes more convincing when their geographical distributions are plotted and studied. Each species is confined to a distinct drainage area and river system. A distribution map of the species was published by FRIEDMAN and GORDON (1934). These workers have also pointed out that *P. variatus*

has fifty chromosomes whereas all the other members of the Xiphophorini have forty-eight. These determinations were made from male specimens and represent the diploid numbers of chromosomes.

In interspecific crosses between *P. xiphidium* and *P. maculatus* and between *P. variatus* and *P. maculatus*, evidences of physiological incompatibility appear. Melanotic tumors, neoplastic in nature, appear when spotted (*Sp*) *P. maculatus* are used in interspecific crosses. These neoplastic reactions parallel those seen in *P. maculatus—X. hellerii* intergeneric hybrids. The data on this phase of incompatibility of species collected in this laboratory have not been published but abstracts by REED, GORDON and LANSING, (1933) and GORDON, (1933) report the conditions briefly. STERN (1936) has recently reviewed again the many types of incompatibility and the various degrees of sterility that often result in hybrids of interspecific crosses. Readers of the work on the sex problems involved in Platypoecilus—Xiphophorus crosses, as reported chiefly by KOSSWIG, must be impressed with the complexity which hybridization as a factor introduces into the problems of sex determination.

Granting that the data on species crosses in Platypoecilus may be interpreted on the basis of the XY (male) XX (female) type of sex inheritance, there still remains the possibility that *P. variatus* and *P. xiphidium* may have the ZZ (male), ZW (female) type of sex inheritance within their own species. KOSSWIG and BELLAMY have utilized the XY concept successfully to explain their data on Platypoecilus species hybrids. In our laboratory a small body of data on hybrids may be explained satisfactorily by assuming the WZ type of sex inheritance. When a male *P. maculatus* was crossed with a female *P. variatus* we obtained 15 males and 20 females.

The need is great for study of the genetic behavior of the many variable color patterns in *P. variatus* alone and in *P. xiphidium* alone. The patterns available in these species were briefly mentioned by GORDON (1933). Some of these patterns, particularly the spotted ones with macromelanophores, may be controlled by a sex-linked factor as in the case of the spotted factor in *P. maculatus* (GORDON 1927). It is desirable that the sex determining mechanism be studied within each species, in pure cultures, so to speak. An accurate estimate of the type of sex determination within a species is difficult enough. FRASER and GORDON (1929) pointed out that if one were to cross a spotted and red platyfish, a 'rubra' that carries the *Sp* and *R* on the Z and W chromosomes, as (Z)*R Sp* (W)*R Sp*, to a recessive male (Z)*r sp* (Z)*r sp*, one would obtain both in F_1 and F_2 data which could be interpreted on the basis of XY type of sex inheritance. How much more complicated would be the data if additional complexities inherent in species crosses were superimposed upon the difficulties mentioned.

The diagram presented by BREIDER (1935) represents Xiphophorus as a species in which sex is phenotypically determined. Phenotypic sex determination, I infer, refers to the absence of dominating sex-determining factors in the sex chromosomes. In some strains of Xiphophorus genetic sex factors have been detected which express their sex determining powers in the direction of the ZZ male- ZW female type of sex determination. The culmination of this tendency for the concentration of sex factors in the sex chromosomes in a stable ZZ-ZW manner is seen in *Platypoecilus maculatus*, a close relative of *Xiphophorus hellerii*.

In another cyprinodont fish, *Limia caudofasciata* of Jamaica, BREIDER has indicated that the sex determining mechanism is more or less like that in Xiphophorus but the tendency is toward the concentration of sex determining factors according to the XY male- XX female system. In *Limia nigrofasciata* of Haiti, the sex determining mechanism is, according to BREIDER, definitely XY-XX.

The story presented by various authors of the state of the sex chromosome mechanism in the cyprinodont fishes is intriguing. From a rather indifferent sex state, such as is found in *Xiphophorus hellerii* and in *Limia caudofasciata*, where both male and female sex may be represented by XX and ZZ, specialization has taken place in the heterochromosome in the males of *Limia nigrofasciata*, Lebistes and Aplocheilus to the XY type, and in the females of *Platypoecilus maculatus* to the WZ type. Experimental evidence shows that the specialization of the heterochromosome may be reversed to the indifferent state in Lebistes and Aplocheilus by selection and in Platypoecilus by hybridization with Xiphophorus. These reversals are unstable and upset normal sex ratios, but further study of them may in time provide the details by which the evolution of the two types of sex determination in fishes has come about.

HANS BREIDER'S 1936 paper arrived too late for inclusion in this discussion.

SUMMARY

1. *Platypoecilus maculatus*, the Mexican common platyfish, has the ZW (female) and ZZ (male) type of sex inheritance.

2. The W chromosome as well as the Z may carry dominant factors.

3. The gene *N* for black band ('nigra' of BELLAMY or black platy) is dominant and sex-linked, as well as *R* red body color (red platy) and *Sp*, spotted (spotted platy).

4. Triple dominant females (Z)*N r sp* (W)*n R Sp* and triple dominant males (Z)*N r sp* (Z)*n R Sp* have been obtained.

5. The rate of crossing over between Z and W is approximately one percent and between Z and Z about one half percent.

6. *R* is not allelic to *Sp*.

7. *N* is not allelic to *Sp*.

8. The sex inheritance mechanism of Platypoecilus is stable.

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DEVELOPMENT OF EYE COLORS IN DROSOPHILA: TIME OF ACTION OF BODY FLUID ON CINNABAR[1]

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THE development of vermilion eye color in mosaics of *D. simulans* is not always autonomous (STURTEVANT 1932). By transplantation experiments BEADLE and EPHRUSSI (1936) have shown that the vermilion and cinnabar eye colors of *D. melanogaster* are not autonomous in development. Vermilion and cinnabar late larval eye discs develop the wild type pigment when transplanted to late wild type larvae. Vermilion discs also develop the wild type pigmentation when implanted in cinnabar hosts, but cinnabar discs remain cinnabar in color when developing in vermilion hosts. From this and similar data BEADLE and EPHRUSSI have postulated the presence in the wild type host of two diffusible substances (v^+ and cn^+ substances), the presence of only one of these (the v^+ substance) in the cinnabar tissue, and the absence of both of these substances in the vermilion tissue.

It is of interest to ascertain when these two genetically determined diffusible substances are present and during what period of development they operate in the determination of the ultimate eye pigment. This has been done for the v^+ substance (EPHRUSSI, CLANCY, and BEADLE 1936; BEADLE, CLANCY, and EPHRUSSI 1937). They have shown by the injection of body fluid from wild type donors of various ages into apricot vermilion (w^a v) hosts 60 hours after puparium formation that the v^+ substance was not present in the wild type larvae 3 to 12 hours before puparium formation, but was present in the wild type from 3 to 80 hours after puparium formation. Similarly, body fluid obtained from wild type donors 60 hours after puparium formation and injected into apricot vermilion hosts of various ages effected a change in eye color in all hosts from late larvae to 64 hours old pupae, with a negative or weak effect at 70 hours, and negative results in still older recipients. These results demonstrate the absence of the v^+ substance in the wild type larval body fluid and its presence during approximately four fifths of the prepupal and pupal periods. The data also indicate the possibility of inducing a change in eye color from vermilion toward wild type by the injection of wild type body fluid into w^a v hosts at any time prior to approximately 70 hours after puparium formation.

The following experiments were performed to obtain similar information regarding the cn^+ substance; that is, the time during which it is present in

[1] Work done at the Institut de Biologie Physico-Chimique, Paris.

the body fluid of the wild type, and the period during which its introduction into cinnabar individuals could cause a change in their eye color. In the work of EPHRUSSI, CLANCY, and BEADLE and in our experiments, some living cells of the wild type donor were undoubtedly introduced with its body fluid into the host. The presence of these chance living cells raised a serious question in the interpretation of the results. To answer this question we have performed additional experiments using fluid from donors killed by heat and by extremely low temperature.

MATERIALS AND METHODS

In these experiments we used the same wild type Oregon-R-c, w^a *v*, and w^a *cn* stocks which were used in the work mentioned above. The rearing of the animals and the injection technique were those employed by EPHRUSSI and BEADLE 1936, and BEADLE, CLANCY and EPHRUSSI 1937. The age of the pupae was determined from the time of puparium formation, puparia being collected at 1 hour intervals. Controls for the injected larvae consisted of uninjected sibs; controls for the injected pupae were sib pupae pricked with an empty injection needle. Such controls were used regularly to determine whether any change in the eye color of the experimental hosts had occurred. Injected or pricked pupae rarely emerge and show only a partial eclosion of the head; these individuals were removed from their cases at the normal time of emergence as adult flies. All the experiments were performed at 25°C.

EXPERIMENTAL RESULTS

Time of presence of the cn^+ substance

The first series of experiments was performed to determine the time during which the cn^+ substance was present in the body fluid of the wild type donor. Body fluid from the wild type donors of known age was injected into apricot-cinnabar larvae that were close to puparium formation. The double recessives apricot-vermilion and apricot-cinnabar have been found much more sensitive indicators of the presence of the v^+ and cn^+ substances than the simple recessives vermilion and cinnabar (BEADLE and EPHRUSSI 1936). The results are shown in table 1. The first column gives the age in hours from puparium formation of the wild type donor, the second column the number of w^a *cn* recipients whose eye color was definitely shifted toward that of the normal allelomorph of *cn*, the third the number of injected flies in which the change of eye color was questionable, the fourth column the number of unaffected individuals, and the last column the total number of larvae that survived the injection and pupation and emerged as adult imagos.

The body fluid of wild type larvae close to puparium formation produced

no change in the eye color of the w^a *cn* recipients. Apparently the cn^+ substance was not present in the body fluid of the late larvae, nor in the chance cells injected with that fluid; nor was it formed by these cells later in the host. Wild type body juice obtained during the first 70 hours fol-

TABLE 1

Effect of wild type pupal fluid injected into w^acn 3-day larvae.

AGE OF DONOR IN HOURS	EFFECT ON HOST				INTENSITY OF EFFECT
	POSITIVE	QUESTIONABLE	NEGATIVE	TOTAL	
Late larvae	0	0	12	12	
Pupae					
1:10– 2:25	3	0	3	6	2 strong, 1 slight
5:30– 6:50	2	2	0	4	1 strong, 1 slight
16:30–19:15	4	0	0	4	4 strong
25:00–41:45	4	3	3	10	1 strong, 3 slight
40:00–46:00	12	1	0	13	11 strong, 1 slight
54:00–69:45	7	1	5	13	6 strong, 1 slight
71:15–72:30	1	0	1	2	slight
78:00–82:00	3	0	9	12	slight
89:45–92:00	1	9	17	27	slight
94:45–99:15	4	1	14	19	slight

lowing puparium formation and injected into w^a *cn* larvae produced a marked change in a large proportion of the surviving individuals. Body fluid drawn from wild type donors during the last 30 hours of the pupal period produced only a slight but certain effect on the pigmentation of the recipients' eyes, and this effect was obtained in a relatively small proportion of the treated animals. The cn^+ substance was present in the wild type body fluid in a high concentration for the first 0.7 of the prepupal and pupal periods, and in a low concentration thereafter until the eclosion o the adult.

The duration of the period when the cn^+ substance is present in the wild type body fluid differs from that found for the v^+ substance (EPHRUSSI, CLANCY, and BEADLE 1936; BEADLE, CLANCY, and EPHRUSSI 1937). These authors found no indication of the presence of the v^+ substance in the wild type body fluid after 80 hours from puparium formation. However, their tests were made by injection into 60 hours old w^a *v* pupae, while we used w^a *cn* larvae in testing for the presence of the cn^+ substance. We injected body fluid from wild type pupae $94\frac{1}{2}$–$97\frac{1}{2}$ hours old (close to emergence) into w^a *v* larvae and obtained definite changes in the eye color of 9 out of 11 recipients. Apparently larval hosts are more sensitive than pupal hosts as indicators of slight amounts of the v^+ and cn^+ substances. These two substances were present in the body fluid of the wild type in-

dividuals from shortly after puparium formation until close to the time of eclosion.

Time of effective-period of the cn⁺substance

Since these two substances are present during practically the entire prepupal and pupal periods in the wild type, their effective-periods in the pupae are of considerable theoretical interest. Since we have used the wild type Oregon-R-c stock while BEADLE, CLANCY, and EPHRUSSI had used the Florida wild type stock as donors in the determination of the effective-period for the v^+ substance, we considered it advisable to re-

TABLE 2

Effect of wild type pupal fluid injected into $w^a cn$ pupae.

AGE OF HOST IN HOURS FROM PUPARIUM FORMATION	EFFECT ON HOST				INTENSITY OF EFFECT
	POSITIVE	QUESTIONABLE	NEGATIVE	TOTAL	
22:30–23:45	6	0	0	6	6 strong
50:00–51:10	8	0	1	9	2 strong, 6 slight
60:20–61:30	6	2	2	10	5 strong, 1 slight
63:30–64:45	12	0	3	15	3 strong, 9 slight
64:00–65:15	12	0	0	12	2 strong, 10 slight
65:15–66:30	10	1	4	15	4 strong, 6 slight
66:40–67:50	13	0	2	15	5 strong, 8 slight
68:00–69:45	21	0	9	30	extremely slight
71:10–73:15	11	3	9	23	extremely slight
76:00–77:00	3	0	13	16	extremely slight
89:30–90:15	9	0	0	9	extremely slight
91:00–92:00	7	0	0	7	extremely slight

examine the total effective-period of the v^+ substance. The following experiments were performed to determine, with a reasonable degree of certitude, the termination of the effective-period of the v^+ and the cn^+ substances when introduced into vermilion and cinnabar recipients. In this work it was impossible to determine the time at which the reaction began because the substance may be retained and work later. The double recessives w^a *v* and w^a *cn* were used as hosts because of their more sensitive reaction, and parallel experiments were performed simultaneously with recipients of equivalent ages. The donors were wild type sib pupae collected during the same hour for injection of their body fluid into w^a *v* and w^a *cn* hosts of the same age.

The data on the effective-period for the cn^+ substance are shown in table 2. The body fluid of wild type pupae was obtained from donors 30–50 hours after puparium formation, the major portion of the work being done with 30–40 hours old donors. The fluid was injected into w^a *cn* hosts of the indicated ages in hours from the time of puparium formation. From tables

1 and 2 it is obvious that a modification of the host eye color toward the wild type was obtained when the cn^+ substance was introduced into individuals lacking it (genotype cinnabar) at any time from the late larval period until approximately 10 hours before eclosion. But there was a sharp break in the degree of eye color change between the group of w^a *cn* pupal hosts aged 66:40 to 67:50 hours and those aged 68 to 69:45 hours. The tests from 50 to 77 hours were run simultaneously allowing comparison between the animals obtained for each of the time intervals indicated in table 2. Pupal hosts 67 hours old or younger at the time of injection

TABLE 3

Effect of wild type pupal fluid injected into $w^a v$ pupae.

AGE OF HOST IN HOURS FROM PUPARIUM FORMATION	EFFECT ON HOST				INTENSITY OF EFFECT
	POSITIVE	QUESTIONABLE	NEGATIVE	TOTAL	
50:00–51:00	6	0	0	6	6 strong
60:00–61:20	14	0	1	15	12 strong, 2 slight
64:00–65:00	9	0	0	9	5 strong, 4 slight
68:00–69:15	12	0	1	13	slight
72:00–73:10	7	0	3	10	slight
76:00–77:00	10	0	0	10	slight
88:20–89:30	11	0	0	11	extremely slight
91:00–92:00	9	0	0	9	extremely slight

showed marked changes in eye color of many of the animals; in hosts 68 hours of age or older positive effects were obtained, but in every case they were extremely weak (detectable with certainty only when the eyes of the controls and experimental animals were placed under water and compared; a definite but delicate yellow could then be distinguished in the host's eyes which was not present in the eyes of the controls). It is evident that the major reaction in the eye pigment formation involving the cn^+ substance ends about 68 hours after puparium formation.

The results for the v^+ substance are shown in table 3. Positive effects were obtained again throughout all the periods tested. Here also there was a definite change in the intensity of the effect. Hosts through 61:20 hours showed pronounced or clear effects from the injections, hosts 64–65 hours showed fewer strong effects with some individuals only slightly affected (on the whole they showed rather less effect than those 2 hours earlier); beyond 68 hours the recipients showed only weak or slight effects. There was a marked reduction in the capacity to use the introduced v^+ substance at about 65 hours after puparium formation or shortly thereafter, indicating the end of the major effective-period.

Heating and freezing experiments

Throughout the above experiments injections were made with larval and pupal fluids which undoubtedly contained many living cells. This raised a serious question which has already been noted by EPHRUSSI, CLANCY, and BEADLE: were the effects obtained from the injections due to chemical compounds present at that time in the body fluid, or to the subsequent activity of chance cells injected with the fluid? To answer this question two different experiments were performed to kill the cells of the donor prior to the injections. If an effect was then obtained by the injections it might safely be assumed that the substances under consideration were already formed and present at the time of injection.

In the first experiment wild type pupae 31 hours old were sealed in a small thin walled glass ampule and immersed in a water bath at 60°C for 30 minutes. Part of the pupae were saved as controls. Since there was no further development in these controls, it was obvious that they had been killed by subjection to 60°C for 30 minutes. The body fluid of the rest of the treated pupae was injected into w^a *v* larvae. These hosts showed marked changes in their eye color, the change in color being more than half way toward that of the normal allele. Obviously the effect was not due to subsequent activity of chance cells injected with the body fluid.

In the second series wild type pupae were placed in a small vial and immersed in liquid air at about −190°C., where they were frozen. Then the vial was removed from the liquid air and the pupae were allowed to thaw. This rapid freezing at an extremely low temperature and thawing at room temperature was repeated four times. The body fluid of these dead pupae was then injected into w^a *v* and w^a *cn* larvae. Fluid from pupae 44 hours old effected a definite change in 10 of the 11 w^a *v* hosts which emerged, and fluid from 48–73 hours old donors in 15 out of 18 individuals. Fluid from pupae 1 to 2 hours old produced effects in 8 out of 9 w^a *cn* hosts which emerged, and fluid from 43 hours old pupae caused marked changes in the eye color of 10 of the 14 emerging recipients. Since the treatment killed the cells of the donors, the v^+ and cn^+ substances must have been present at the time of the injections. The two experiments demonstrate: 1) that the substances examined in this work had been formed by the wild type donors prior to the moment their body fluid was withdrawn for injection; 2) that they were apparently stable between approximately −190°C. and +60°C. (EPHRUSSI and HARNLY, 1936).

DISCUSSION

The data demonstrate that the cn^+ substance was not present in the wild type late larvae. This substance was first formed in detectable quantities at about the time of puparium formation, its presence being evi-

denced by changes in the eye color of w^a *cn* hosts injected with body fluid taken from wild type individuals one hour after puparium formation. The concentration of the cn^+ substance was quite low at this time, the changes in eye color being definite but not marked. The concentration apparently increases rapidly and in 18 hour pupae has reached a high concentration, since the amount injected changed the host mutant eye half way or more toward the color of the normal allele. Thereafter relatively large amounts of the cn^+ substance were present in the wild type body fluid as demonstrated by the degree of change in the recipient's eye color and the proportion of individuals affected. This high concentration was maintained until approximately 70 hours after puparium formation, that is, until about 18 hours after the first appearance of eye color in the wild type pupae according to SCHULTZ (1935). The body fluid of wild type pupae 70–99 hours old produced definite but only minor changes when injected into w^a *cn* larvae, and these changes occurred in a relatively small proportion of individuals, indicating that during this period the cn^+ substance was present in a low concentration. EPHRUSSI, CLANCY, and BEADLE (1936) and BEADLE, CLANCY, and EPHRUSSI (1937) have found that the v^+ substance was not present in wild type larval body fluid, but was present in the body fluid shortly after puparium formation, reached a maximum concentration between 8 and 17 hours, and thereafter showed a gradual decrease in effective power becoming undetectable in donor pupae over 80 hours old. Repeating these experiments with injections into larvae instead of pupae, we have found that traces of the v^+ substance are present even later in the wild type body fluid. Both the v^+ and the cn^+ substances seem to appear at approximately the same moment in the development of the wild type individuals. A comparison of our results with those of EPHRUSSI, CLANCY, and BEADLE seems to indicate that there is a difference in the time of fall of the high concentration of these substances; this difference may be real or may be due to the difference in the age of the recipients in the two sets of experiments.

It is difficult to define sharply the effective period for the cn^+ substance. Wild type pupal fluid from a period known to contain a high concentration of the cn^+ substance may produce marked changes in the host's eye color when injected into late w^a *cn* larvae or into pupae until 67 hours after puparium formation. But, since the substance supplied by injection might be retained by the recipient and used later, it was impossible to determine by the technique employed the time at which this substance began to be used by the host. It may be that the inception of the effective-period coincided with the first appearance of the cn^+ substance in effective concentration shortly after puparium formation, but this point is at present undetermined. The end of the major effective-period for the cn^+ substance

clearly falls between 67 and 68 hours after puparium formation and close to the first appearance of color in the eyes of cinnabar pupae. EPHRUSSI, CLANCY, and BEADLE (1936) have found that wild type body fluid containing the v^+ substance when injected into w^a v late larvae or into pupae until about 65 hours after puparium formation may have a marked effect on the recipient's eyes. In the case of the v^+ substance the major effective-period also closes before the first appearance of eye color in the vermilion pupae.

An hypothesis has been developed from the results of the transplantation of eye discs between wild type, cinnabar, vermilion, and claret (BEADLE and EPHRUSSI 1936, BEADLE and EPHRUSSI 1937). "Such an hypothesis assumes that the ca^+, v^+, and cn^+ substances are successive products in a chain reaction. The relation of these substances can be indicated in a simple diagrammatic way as follows:

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance.}"$$

From the work reported here the v^+ and cn^+ substances were apparently formed at about the same time in the development of the wild type individuals. This would be expected if they represent a chain reaction, the formation of the cn^+ substance being dependent on the presence of the v^+ substance and formed by a reaction occurring immediately after its appearance. The apparent difference in the time of fall of the high concentration of these two substances in the wild type, if real, is also in harmony with the assumption that the v^+ substance is formed first and, in a chain reaction, the cn^+ substance appears and is used later. If the difference of two or three hours in the termination of the major effective-periods for the v^+ and the cn^+ substances in w^a v and w^a cn hosts is real it is also in complete agreement with the ca^+, v^+, cn^+ chain hypothesis.

Apparently not all of the v^+ substance is used in the formation of the cn^+ substance nor all of the latter in the subsequent formation of the wild type eye pigment since effective traces of both are still present in the pupae shortly before the emergence of the flies. It is impossible to tell at present whether or not the subsequent darkening of the adult eyes with age is due to the later utilization of the last traces of these and similar substances. It has been indicated earlier in this paper that there was some use of the substances within ten hours of eclosion of the adult w^a v and w^a cn flies. It may be that the so-called "age effects" are merely the continuation or completion of processes going on before emergence.

SUMMARY

1. The body fluid of wild type late larvae does not contain the cn^+ substance (a compound capable of modifying the eye color of the w^a cn host).

2. There is a high concentration of the cn^+ substance in the wild type body fluid from 1 to 70 hours after puparium formation, the high concentration ending about 18 hours after the first appearance of eye color in the wild type pupae. It is present in a low concentration thereafter until the emergence of the adult fly. As demonstrated by BEADLE, CLANCY, and EPHRUSSI the v^+ substance also appears in the wild type individuals shortly after puparium formation, and as shown by the present experiments, can be found in a low concentration in the body fluid as late as 94 to 97 hours after puparium formation.

3. The major effective-period of the cn^+ substance introduced into w^a *cn* hosts ends between 67 and 68 hours after puparium formation, which is close to the first appearance of color in the eyes of cinnabar pupae. As previously reported by BEADLE, CLANCY, and EPHRUSSI, and confirmed here, the major effective-period of the v^+ substance introduced into w^a *v* hosts ends about 65 hours after puparium formation or shortly thereafter, that is, before the first appearance of eye color in the vermilion pupae.

4. Experiments in which chance cells introduced with the body fluid of the wild type donors were killed by heat or freezing demonstrated: that the v^+ and cn^+ substances had been formed previous to injection into w^a *v* and w^a *cn* hosts; and that the v^+ and cn^+ substances were stable between approximately $-190°$C. and $+60°$C.

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THE RELATIVE ROLES OF STABILIZATION AND REDIFFERENTIATION OF THE GENE IN THE EVOLUTION OF THE HEREDITARY SUBSTANCE

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AS SHOWN by a previous investigation (BERG 1937) the mutability of the X chromosome genes to lethals (excluding semilethals) is one third of that of the genes of the second chromosome. It was also demonstrated that this result can probably be explained on the assumption that a certain part of the X chromosome genes have lost their capacity of determining the viability of the individual and consequently the capacity of mutating towards alleles which reduce viability. These genes have turned into sex determiners, as shown by the high frequency of sterility mutations in the X chromosome as compared with that in the second chromosome.

The process of redifferentiation, however, does not exclude another process—the stabilization of the gene with respect to injurious effects.

The present paper deals with an attempt to throw some light on the relative rôles of these processes in the evolution of the hereditary material.

The assumption at the beginning of this work was as follows. If the stabilization process does not play a considerable rôle in the evolution of the X chromosome genes, the difference observed for respective mutabilities of the X and the second chromosome towards lethals (1:3) ought to be explained entirely by the redifferentiation of the X chromosome genes towards sex determination. In this case the sterility mutations must be at least twice as numerous as lethal mutations in the X chromosome (2:1) because two thirds of the original normal alleles of lethals would have become normal alleles of sterility genes. A lower ratio than this would indicate the existence of another process besides the redifferentiation process—possibly stabilization. The amount of reduction will also show the degree of influence of this second process upon the lower mutability of the X chromosome genes as compared to the autosomal genes.

The present experiment was undertaken also to establish the relation between the amount of decrease of viability caused by a mutation and the existence of a sterility effect. Many genes that have not entirely lost influence upon viability may at the same time be on the way to becoming sex determiners.

THE EXPERIMENT

The experiment undertook a comparison of lethal and sterility mutations in the X chromosome of *D. melanogaster*. The detection of lethals

and semilethals was done by counting males and females in F_2 cultures (90–200 flies per culture), so that all the degrees of the reduction of viability could be taken into account.

Males w^a were irradiated with a heavy dosage (8000 r) and crossed to ClB/v females. In F_1 the ClB/w^a virgin females were crossed individually to a number of sc^{a1} males. Of these F_1–F_2 cultures, 850 were started and carried through three broods of 3–4 days each. We exclude here all cases in which the female failed to live for this length of time. Of these, 827 proved to be fertile and 23 sterile. (Thus at the most 2.7 percent of dominant sterility mutations in all chromosomes was obtained.)

In F_2 cultures the number of males and females was counted. The markers (v and sc^{a1}, which is connected with two inversions entirely suppressing crossing over in the X chromosome) made it possible to distinguish between all the types of non-disjunction, primary as well as secondary.

TABLE 1

F_2 cultures classified according to percentage of males.

	80<	80–70	70–60	60–50	50–40	40–30	30–20	20–10	10–0	0	TOTAL
Number of F_2 cultures	2	4	20	67	146	100	29	19	22	66	475
Percentage of cultures with the given male viability	*0.4*	*0.9*	*4.4*	*14.3*	*30.7*	*21.1*	*6.1*	*4.2*	*4.8*	*13.9*	
Number of sterility mutations in each class	—	—	—	8	17	17	11	8	6	—	67
Percentage of sterility mutations in each class	—	—	—	*11.9*	*11.6*	*17*	*37.9*	*42.1*	*27.3*	—	

We thus examined 475 F_2 cultures. Among them there were 66 lethals (13.9 percent). Only those cases were considered as lethals in which not a single male was observed either in the original F_1–F_2 cultures or in the F_2–F_3 cultures derived from them.

The non-lethal cultures (408) were tested for sterility mutations by crossing all F_2 males in a group to their sisters; 52 cases of complete sterility were observed (12.7 percent) and 15 cases with a very great reduction of fertility.

All mutations, lethals, semilethals and sterility mutations, were tested in the next generation.

The sterility mutations were distributed among the cultures of F_2 with different degrees of viability reduction as shown in table 1.

DISCUSSION

The experiment showed that the number of sterility mutations (12.7 percent) does not exceed the number of lethal mutations (13.9 percent). This ratio is very near to 1:1.

Consequently the redifferentiation of genes cannot be the single cause of the small number of lethal mutations arising in the X chromosome as compared to autosomes. The stabilization processes must have had their part in the evolution of the X chromosome genes and this part was not less significant than that of the redifferentiation processes. This conclusion is drawn from the comparison of the ratio obtained (1:1) with the ratio expected (2:1) when the stabilization factor is excluded.

It would be of course true only if the number of sterility mutations indicates the number of genes affecting sex. It is possible, however, that some genes which do not affect viability, affect sex so little that a mutation of one of these would not produce complete sterility.

But these genes cannot be numerous, for they would be eliminated by natural selection in a way similar to the Y chromosome genes (MULLER and PAINTER, 1932) as their mutation would have almost no phenotypical expression.

It is evident from the data of table 1 that the decrease of viability is to some extent connected with the increase of the frequency of sterility mutations among the cultures of the corresponding class. This fact verifies the suggestion that many genes affecting viability have a pleiotropic effect, acting also as sex determiners.

Among those cultures, however, which show the most pronounced reduction of viability, a considerable decrease of sterility mutations should be expected. In these cultures cases are met with, when a slight mutation of a gene playing an essential rôle in vital processes of the organism occurs. Even a very slight mutation of such a gene can result in a considerable decrease of viability. A gene of this sort would be less likely to be subjected to a redifferentiation process. The latter might be expected rather for genes having a subordinate significance for viability. Such genes must be more labile (mutable) and their mutation would often be connected with sterility.

Pleiotropy of the gene may be an intermediate step in its change of function.

The decrease in the proportion of sterility mutations in cultures with a great decrease of viability can in fact be observed in this experiment, but unfortunately this question can not be answered owing to the insufficiency of the material. Further data of this kind, as well as a morphological study of the manifestation of sterility mutations should throw some light on this question.

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The author expresses to Professor H. J. MULLER her gratitude for supervision and help in this work and to Professor A. P. VLADIMIRSKY for his interest and criticism.

SUMMARY

1. A hypothesis is proposed that genes for viability in the X chromosomes redifferentiate into those concerned with sex differentiation.

2. The frequency of sterility mutations in the X chromosomes, although relatively high as compared with that in autosomes, is not high enough fully to explain the much lesser frequency of lethal mutations in the X as compared with autosomes, on the above hypothesis.

3. Besides the above redifferentiation processes there must be a process of gene stabilization.

4. The percent of sterility mutation is higher among cultures showing a decrease of viability; this indicates the pleiotropic action of the many X chromosome genes affecting such relatively independent features of the organism as fertility and viability.

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CONTENTS

Vol. XII - N. 4 30-IX-1936

C. GINI. Dell' influenza che il raggruppamento delle singole modalità esercita sul valore di alcuni indici statistici nel caso di serie sconnesse ..... Pag. 3

H. KŒPPLER. Das Wahrscheinlichkeitsgesetz zweier wahrer einander zugeordneten Fehler und einige mit diesem zusammenhängende Betrachtungen ... " 35

S. KULLBACK. A note on the multiple correlation coefficient ..... " 67

S. KOLLER. Die Analyse der Abhaengigkeitsverhaeltnisse in zwei Korrelationssystemen ..... " 73

G. PIETRA. Andreas M. Andréadès, Carl V. L. Charlier, Emanuel Czuber, George Knibbs, Lucien March. „In Memoriam" ..... " 107

Bibliografia ..... " 121

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CONTENTS OF PRECEDING ISSUES

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